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Harosh et al.

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(54) **BOROPEPTIDE INHIBITORS OF ENTEROPEPTIDASE AND THEIR USES IN TREATMENT OF OBESITY, OVERWEIGHT AND/OR DISEASES ASSOCIATED WITH AN ABNORMAL FAT METABOLISM**

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(51) **Int. Cl.**

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C07D 517/00	(2006.01)
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C07K 5/062	(2006.01)
C07K 5/065	(2006.01)
C07K 5/072	(2006.01)
C07K 5/083	(2006.01)
C07K 5/087	(2006.01)
C07K 5/09	(2006.01)
A61K 38/05	(2006.01)
A61K 38/06	(2006.01)
C07K 5/06	(2006.01)
A61K 38/00	(2006.01)

(52) **U.S. Cl.**

CPC ***C07K 5/08*** (2013.01); ***A61K 38/005*** (2013.01); ***A61K 38/05*** (2013.01); ***A61K 38/06*** (2013.01); ***C07K 5/06*** (2013.01); ***C07K 5/0606*** (2013.01); ***C07K 5/06026*** (2013.01); ***C07K 5/06043*** (2013.01); ***C07K 5/06078*** (2013.01); ***C07K 5/06095*** (2013.01); ***C07K 5/06104*** (2013.01); ***C07K 5/06191*** (2013.01); ***C07K 5/0806*** (2013.01); ***C07K 5/0812*** (2013.01); ***C07K 5/0815*** (2013.01); ***C07K 5/0817*** (2013.01); ***C07K 5/0827*** (2013.01); *A61K 38/00* (2013.01)

(58) **Field of Classification Search**

CPC A61K 38/00; A61K 38/005; A61K 38/05; A61K 38/06; C07K 5/06; C07K 5/06026; C07K 5/06043; C07K 5/0606; C07K 5/06078; C07K 5/06095; C07K 5/06104; C07K 5/08; C07K 5/0806; C07K 5/0812

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,867,539	A	2/1975	Henkin
4,446,138	A	5/1984	Pack
4,588,724	A	5/1986	Greenway, III et al.
4,745,122	A	5/1988	Lassen
5,019,594	A	5/1991	Wurtman et al.
5,187,157	A	2/1993	Kettner et al.
5,300,298	A	4/1994	LaNoue
5,403,851	A	4/1995	D'Orlando et al.
5,567,714	A	10/1996	Bruns, Jr. et al.
5,573,774	A	11/1996	Keenan
5,578,613	A	11/1996	Bryant et al.
5,814,622	A	9/1998	de Nanteuil et al.
5,900,411	A	5/1999	Morfeldt
6,066,650	A	5/2000	Biller et al.
6,121,283	A	9/2000	Chang et al.
6,369,075	B1	4/2002	Ruggeri et al.

FOREIGN PATENT DOCUMENTS

EP	0293881 A2	12/1988
WO	9509634 A1	4/1995
WO	2006050999 A2	5/2006

OTHER PUBLICATIONS

Of Kettner et al, The Journal of Biological Chemistry, vol. 265, No. 30, Issue of Oct. 25, pp. 18289-18297, 1990.*

Abe et al Regulatory Peptides 138 (2007) 56-58.*

Avellone et al, Diabetes Res. 1994; 25(2):85-92.*

Hadorn, B. et al., (1969) Intestinal enterokinase deficiency. Lancet 1(7599):812-813.

Holyoak, T. et al., (2003) 2.4 A resolution crystal structure of the prototypical hormone-processing protease Kex2 in complex with an Ala-Lys-Arg boronic acid inhibitor. Biochemistry 42(22):6709-6718.

(Continued)

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(57) **ABSTRACT**

Novel compounds, particularly derivatives of boroarginine, boroornithine and borolysine that selectively modulate, regulate, and/or inhibit enteropeptidase. Compositions, particularly pharmaceutical compositions, as well as methods to treat excess weight, obesity and diseases associated with an abnormal fat metabolism.

9 Claims, 25 Drawing Sheets

(56) **References Cited**

OTHER PUBLICATIONS

Imamura, T. And Kitamoto, Y. (2003) Expression of enteropeptidase in differentiated enterocytes, goblet cells, and the tumor cells in human duodenum. Am J Physiol Gastrointest Liver Physiol. 285(6):G1235-1241.

Katz, B. A, et al., (1995) Episelection: novel Ki approximately nanomolar inhibitors of serine proteases selected by binding or chemistry on an enzyme surface. Biochemistry 34(26):8264-8280.

Kettner, C. et al., (1990) The selective inhibition of thrombin by peptides of boroarginine. J Biol Chem 265 (30):18289-18297.

Komiyama, T. et al., (2005) Protection from anthrax toxin-mediated killing of macrophages by the combined effects of furin inhibitors and chloroquine. Antimicrob Agents Chemother 49(9):3875-3882.

Lebarbier, C. et al., (1998) Synthesis of boronic acid analogs of L-arginine as alternate substrates or inhibitors of nitric oxide synthase. Bioorg Med Chem Lett 8(18):2573-2576.

Lentze, M. J. (1982) Intestinal enteropeptidase deficiency associated with exocrine pancreatic insufficiency. Lancet 2 (8296):504.

Mann, N. S. and Mann, S. K. (1994) Enterokinase. Proc Soc Exp Biol Med 206(2):114-118.

Quan, M. L. et al., (1997) Biaryl substituted alkylboronate esters as thrombin inhibitors. Bioorg Med Chem Lett 7 (13):1595-1600.

Roberds, S. L. et al., (2001) BACE knockout mice are healthy despite lacking the primary beta-secretase activity in brain: implications for Alzheimer's disease therapeutics. Hum mol Genet 10(12):1317-1324.

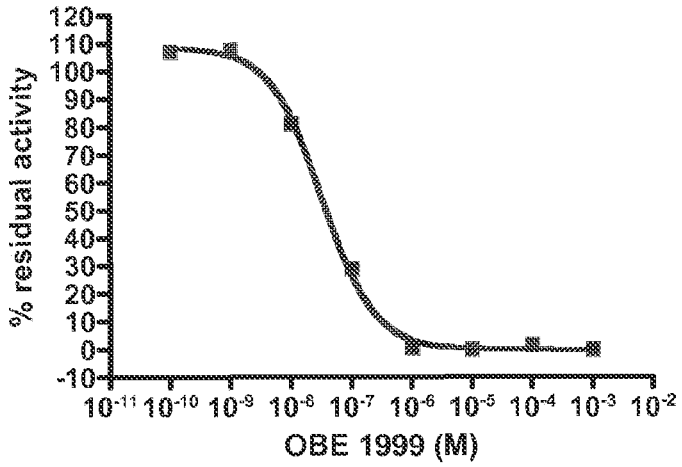
Sharp, D. et al., (1993) Cloning and gene defects in microsomal triglyceride transfer protein associated with abetalipoproteinaemia. Nature 365(6441):65-69.

Stadnicki, A. et al., (1998) Specific inhibition of plasma kallikrein modulates chronic granulomatous intestinal and systemic inflammation in genetically susceptible rats. FASEB J 12(3):325-333.

Wetterau, J. R. et al., (1994) An MTP inhibitor that normalizes atherogenic lipoprotein levels in WHHL rabbits. Science 282(5389):751-754.

Yin, Z. et al., (2006) Peptide inhibitors of Dengue virus NS3 protease. Part 1: Warhead. Bioorg Med Chem Lett 16 (1):36-39.

* cited by examiner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90
80
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OBE 1999 (M)

Fig. 1

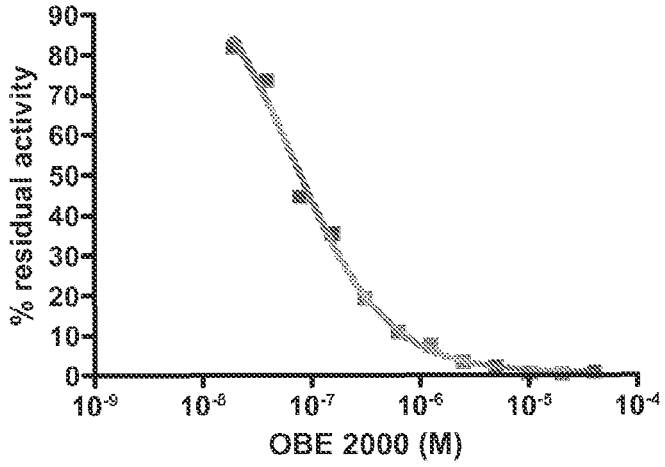
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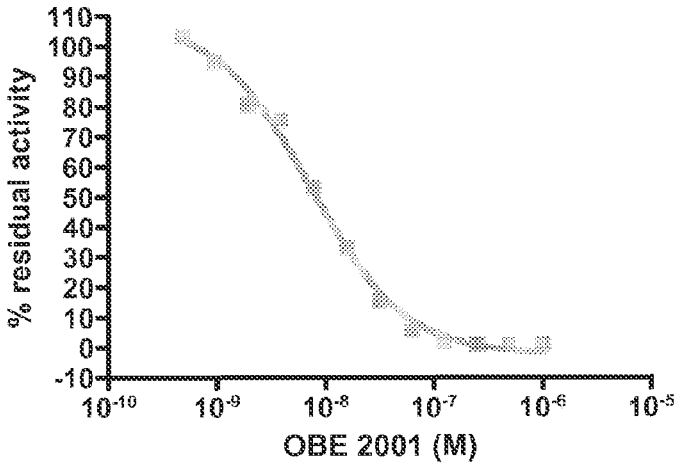
Fig. 2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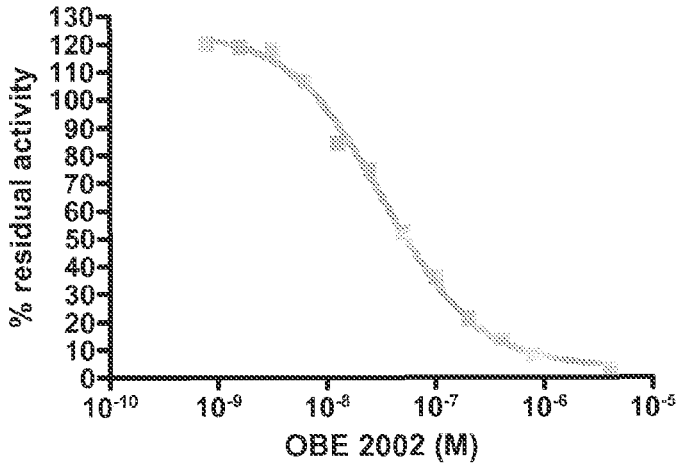
OBE 2001 (M)

Fig. 3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10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
OBE 2002 (M)

Fig. 4

Enzyme	IC 50 (nM)			
	OBE 1999	OBE 2000	OBE 2001	OBE 2002
Enteropeptidase	33.2	68	7.3	33.6
Trypsin	10.8	10.6	157	10.5
Thrombin	6400	3220	4000	4000
Plasmin	29.8	7100	21.7	3800
Kallikrein	7.5	260	6.9	81.4
Chymotrypsin	No inhibition	No inhibition	No inhibition	No inhibition
Elastase	No inhibition	No inhibition	No inhibition	No inhibition
Carboxypeptidase A1	No inhibition	No inhibition	No inhibition	No inhibition
Carboxypeptidase B1	No inhibition	No inhibition	No inhibition	No inhibition
α-amylase	No inhibition	No inhibition	No inhibition	No inhibition
DPPIV	No inhibition	No inhibition	No inhibition	No inhibition

Fig. 5

```
accagacagt tcttaaatta gcaagccttc aaaaccaaaa atg ggg tcg aaa aga        55
                                              Met Gly Ser Lys Arg
                                              1               5

ggc ata tct tct agg cat cat tct ctc agc tcc tat gaa atc atg ttt       103
Gly Ile Ser Ser Arg His His Ser Leu Ser Ser Tyr Glu Ile Met Phe
                10                  15                  20

gca gct ctc ttt gcc ata ttg gta gtg ctc tgt gct gga tta att gca       151
Ala Ala Leu Phe Ala Ile Leu Val Val Leu Cys Ala Gly Leu Ile Ala
            25                  30                  35

gta tcc tgc ctg aca atc aag gaa tcc caa cga ggt gca gca ctt gga       199
Val Ser Cys Leu Thr Ile Lys Glu Ser Gln Arg Gly Ala Ala Leu Gly
        40                  45                  50

cag agt cat gaa gcc aga gcg aca ttt aaa ata aca tcc gga gtt aca       247
Gln Ser His Glu Ala Arg Ala Thr Phe Lys Ile Thr Ser Gly Val Thr
    55                  60                  65

tat aat cct aat ttg caa gac aaa ctc tca gtg gat ttc aaa gtt ctt       295
Tyr Asn Pro Asn Leu Gln Asp Lys Leu Ser Val Asp Phe Lys Val Leu
70                  75                  80                  85

gct ttt gac ctt cag caa atg ata gat gag atc ttt cta tca agc aat       343
Ala Phe Asp Leu Gln Gln Met Ile Asp Glu Ile Phe Leu Ser Ser Asn
                90                  95                  100

ctg aag aat gaa tat aag aac tca aga gtt tta caa ttt gaa aat ggc       391
Leu Lys Asn Glu Tyr Lys Asn Ser Arg Val Leu Gln Phe Glu Asn Gly
            105                 110                 115

agc att ata gtc gta ttt gac ctt ttc ttt gcc cag tgg gtg tca gat       439
Ser Ile Ile Val Val Phe Asp Leu Phe Phe Ala Gln Trp Val Ser Asp
        120                 125                 130

caa aat gta aaa gaa gaa ctg att caa ggc ctt gaa gca aat aaa tcc       487
Gln Asn Val Lys Glu Glu Leu Ile Gln Gly Leu Glu Ala Asn Lys Ser
    135                 140                 145

agc caa ctg gtc act ttc cat att gat ttg aac agc gtt gat atc cta       535
Ser Gln Leu Val Thr Phe His Ile Asp Leu Asn Ser Val Asp Ile Leu
150                 155                 160                 165

gac aag cta aca acc acc agt cat ctg gca act cca gga aat gtc tca       583
Asp Lys Leu Thr Thr Thr Ser His Leu Ala Thr Pro Gly Asn Val Ser
                170                 175                 180

ata gag tgc ctg cct ggt tca agt cct tgt act gat gct cta acg tgt       631
Ile Glu Cys Leu Pro Gly Ser Ser Pro Cys Thr Asp Ala Leu Thr Cys
            185                 190                 195

ata aaa gct gat tta ttt tgt gat gga gaa gta aac tgt cca gat ggt       679
Ile Lys Ala Asp Leu Phe Cys Asp Gly Glu Val Asn Cys Pro Asp Gly
        200                 205                 210
```

Fig. 6A

```
tct gac gaa gac aat aaa atg tgt gcc aca gtt tgt gat gga aga ttt      727
Ser Asp Glu Asp Asn Lys Met Cys Ala Thr Val Cys Asp Gly Arg Phe
    215                 220                 225

ttg tta act gga tca tct ggg tct ttc cag gct act cat tat cca aaa      775
Leu Leu Thr Gly Ser Ser Gly Ser Phe Gln Ala Thr His Tyr Pro Lys
230                 235                 240                 245

cct tct gaa aca agt gtt gtc tgc cag tgg atc ata cgt gta aac caa      823
Pro Ser Glu Thr Ser Val Val Cys Gln Trp Ile Ile Arg Val Asn Gln
                250                 255                 260

gga ctt tcc att aaa ctg agc ttc gat gat ttt aat aca tat tat aca      871
Gly Leu Ser Ile Lys Leu Ser Phe Asp Asp Phe Asn Thr Tyr Tyr Thr
            265                 270                 275

gat ata tta gat att tat gaa ggt gta gga tca agc aag att tta aga      919
Asp Ile Leu Asp Ile Tyr Glu Gly Val Gly Ser Ser Lys Ile Leu Arg
        280                 285                 290

gct tct att tgg gaa act aat cct ggc aca ata aga att ttt tcc aac      967
Ala Ser Ile Trp Glu Thr Asn Pro Gly Thr Ile Arg Ile Phe Ser Asn
    295                 300                 305

caa gtt act gcc acc ttt ctt ata gaa tct gat gaa agt gat tat gtt     1015
Gln Val Thr Ala Thr Phe Leu Ile Glu Ser Asp Glu Ser Asp Tyr Val
310                 315                 320                 325

ggc ttt aat gca aca tat act gca ttt aac agc agt gag ctt aat aat     1063
Gly Phe Asn Ala Thr Tyr Thr Ala Phe Asn Ser Ser Glu Leu Asn Asn
                330                 335                 340

tat gag aaa att aat tgt aac ttt gag gat ggc ttt tgt ttc tgg gtc     1111
Tyr Glu Lys Ile Asn Cys Asn Phe Glu Asp Gly Phe Cys Phe Trp Val
            345                 350                 355

cag gat cta aat gat gat aat gaa tgg gaa agg att cag gga agc acc     1159
Gln Asp Leu Asn Asp Asp Asn Glu Trp Glu Arg Ile Gln Gly Ser Thr
        360                 365                 370

ttt tct cct ttt act gga ccc aat ttt gac cac act ttt ggc aat gct     1207
Phe Ser Pro Phe Thr Gly Pro Asn Phe Asp His Thr Phe Gly Asn Ala
    375                 380                 385

tca gga ttt tac att tct acc cca act gga cca gga ggg aga caa gaa     1255
Ser Gly Phe Tyr Ile Ser Thr Pro Thr Gly Pro Gly Gly Arg Gln Glu
390                 395                 400                 405

cga gtg ggg ctt tta agc ctc cct ttg gac ccc act ttg gag cca gct     1303
Arg Val Gly Leu Leu Ser Leu Pro Leu Asp Pro Thr Leu Glu Pro Ala
                410                 415                 420

tgc ctt agt ttc tgg tat cat atg tat ggt gaa aat gtc cat aaa tta     1351
Cys Leu Ser Phe Trp Tyr His Met Tyr Gly Glu Asn Val His Lys Leu
            425                 430                 435
```

Fig. 6B

```
agc att aat atc agc aat gac caa aat atg gag aag aca gtt ttc caa      1399
Ser Ile Asn Ile Ser Asn Asp Gln Asn Met Glu Lys Thr Val Phe Gln
        440                 445                 450

aag gaa gga aat tat gga gac aat tgg aat tat gga caa gta acc cta      1447
Lys Glu Gly Asn Tyr Gly Asp Asn Trp Asn Tyr Gly Gln Val Thr Leu
    455                 460                 465

aat gaa aca gtt aaa ttt aag gtt gct ttt aat gct ttt aaa aac aag      1495
Asn Glu Thr Val Lys Phe Lys Val Ala Phe Asn Ala Phe Lys Asn Lys
470                 475                 480                 485

atc ctg agt gat att gcg ttg gat gac att agc cta aca tat ggg att      1543
Ile Leu Ser Asp Ile Ala Leu Asp Asp Ile Ser Leu Thr Tyr Gly Ile
                490                 495                 500

tgc aat ggg agt ctt tat cca gaa cca act ttg gtg cca act cct cca      1591
Cys Asn Gly Ser Leu Tyr Pro Glu Pro Thr Leu Val Pro Thr Pro Pro
            505                 510                 515

cca gaa ctt cct acg gac tgt gga gga cct ttt gag ctg tgg gag cca      1639
Pro Glu Leu Pro Thr Asp Cys Gly Gly Pro Phe Glu Leu Trp Glu Pro
        520                 525                 530

aat aca aca ttc agt tct acg aac ttt cca aac agc tac cct aat ctg      1687
Asn Thr Thr Phe Ser Ser Thr Asn Phe Pro Asn Ser Tyr Pro Asn Leu
    535                 540                 545

gct ttc tgt gtt tgg att tta aat gca caa aaa gga aag aat ata caa      1735
Ala Phe Cys Val Trp Ile Leu Asn Ala Gln Lys Gly Lys Asn Ile Gln
550                 555                 560                 565

ctt cat ttt caa gaa ttt gac tta gaa aat att aac gat gta gtt gaa      1783
Leu His Phe Gln Glu Phe Asp Leu Glu Asn Ile Asn Asp Val Val Glu
                570                 575                 580

ata aga gat ggt gaa gaa gct gat tcc ttg ctc tta gct gtg tac aca      1831
Ile Arg Asp Gly Glu Glu Ala Asp Ser Leu Leu Leu Ala Val Tyr Thr
            585                 590                 595

ggg cct ggc cca gta aag gat gtg ttc tct acc acc aac aga atg act      1879
Gly Pro Gly Pro Val Lys Asp Val Phe Ser Thr Thr Asn Arg Met Thr
        600                 605                 610

gtg ctt ctc atc act aac gat gtg ttg gca aga gga ggg ttt aaa gca      1927
Val Leu Leu Ile Thr Asn Asp Val Leu Ala Arg Gly Gly Phe Lys Ala
    615                 620                 625

aac ttt act act ggc tat cac ttg ggg att cca gag cca tgc aag gca      1975
Asn Phe Thr Thr Gly Tyr His Leu Gly Ile Pro Glu Pro Cys Lys Ala
630                 635                 640                 645

gac cat ttt caa tgt aaa aat gga gag tgt gtt cca ctg gtg aat ctc      2023
Asp His Phe Gln Cys Lys Asn Gly Glu Cys Val Pro Leu Val Asn Leu
                650                 655                 660
```

Fig. 6C

```
tgt gac ggt cat ctg cac tgt gag gat ggc tca gat gaa gca gat tgt     2071
Cys Asp Gly His Leu His Cys Glu Asp Gly Ser Asp Glu Ala Asp Cys
            665                 670                 675

gtg cgt ttt ttc aat ggc aca acg aac aac aat ggt tta gtg cgg ttc     2119
Val Arg Phe Phe Asn Gly Thr Thr Asn Asn Asn Gly Leu Val Arg Phe
        680                 685                 690

aga atc cag agc ata tgg cat aca gct tgt gct gag aac tgg acc acc     2167
Arg Ile Gln Ser Ile Trp His Thr Ala Cys Ala Glu Asn Trp Thr Thr
    695                 700                 705

cag att tca aat gat gtt tgt caa ctg ctg gga cta ggg agt gga aac     2215
Gln Ile Ser Asn Asp Val Cys Gln Leu Leu Gly Leu Gly Ser Gly Asn
710                 715                 720                 725

tca tca aag cca atc ttc tct acc gat ggt gga cca ttt gtc aaa tta     2263
Ser Ser Lys Pro Ile Phe Ser Thr Asp Gly Gly Pro Phe Val Lys Leu
                730                 735                 740

aac aca gca cct gat ggc cac tta ata cta aca ccc agt caa cag tgt     2311
Asn Thr Ala Pro Asp Gly His Leu Ile Leu Thr Pro Ser Gln Gln Cys
            745                 750                 755

tta cag gat tcc ttg att cgg tta cag tgt aac cat aaa tct tgt gga     2359
Leu Gln Asp Ser Leu Ile Arg Leu Gln Cys Asn His Lys Ser Cys Gly
        760                 765                 770

aaa aaa ctg gca gct caa gac atc acc cca aag att gtt gga gga agt     2407
Lys Lys Leu Ala Ala Gln Asp Ile Thr Pro Lys Ile Val Gly Gly Ser
    775                 780                 785

aat gcc aaa gaa ggg gcc tgg ccc tgg gtt gtg ggt ctg tat tat ggc     2455
Asn Ala Lys Glu Gly Ala Trp Pro Trp Val Val Gly Leu Tyr Tyr Gly
790                 795                 800                 805

ggc cga ctg ctc tgc ggc gca tct ctc gtc agc agt gac tgg ctg gtg     2503
Gly Arg Leu Leu Cys Gly Ala Ser Leu Val Ser Ser Asp Trp Leu Val
                810                 815                 820

tcc gcc gca cac tgc gtg tat ggg aga aac tta gag cca tcc aag tgg     2551
Ser Ala Ala His Cys Val Tyr Gly Arg Asn Leu Glu Pro Ser Lys Trp
            825                 830                 835

aca gca atc cta ggc ctg cat atg aaa tca aat ctg acc tct cct caa     2599
Thr Ala Ile Leu Gly Leu His Met Lys Ser Asn Leu Thr Ser Pro Gln
        840                 845                 850

aca gtc cct cga tta ata gat gaa att gtc ata aac cct cat tac aat     2647
Thr Val Pro Arg Leu Ile Asp Glu Ile Val Ile Asn Pro His Tyr Asn
    855                 860                 865

agg cga aga aag gac aac gac att gcc atg atg cat ctg gaa ttt aaa     2695
Arg Arg Arg Lys Asp Asn Asp Ile Ala Met Met His Leu Glu Phe Lys
870                 875                 880                 885
```

Fig. 6D

```
gtg aat tac aca gat tac ata caa cct att tgt tta ccg gaa gaa aat     2743
Val Asn Tyr Thr Asp Tyr Ile Gln Pro Ile Cys Leu Pro Glu Glu Asn
                890                 895                 900

caa gtt ttt cct cca gga aga aat tgt tct att gct ggt tgg ggg acg     2791
Gln Val Phe Pro Pro Gly Arg Asn Cys Ser Ile Ala Gly Trp Gly Thr
            905                 910                 915

gtt gta tat caa ggt act act gca aac ata ttg caa gaa gct gat gtt     2839
Val Val Tyr Gln Gly Thr Thr Ala Asn Ile Leu Gln Glu Ala Asp Val
        920                 925                 930

cct ctt cta tca aat gag aga tgc caa cag cag atg cca gaa tat aac     2887
Pro Leu Leu Ser Asn Glu Arg Cys Gln Gln Gln Met Pro Glu Tyr Asn
    935                 940                 945

att act gaa aat atg ata tgt gca ggc tat gaa gaa gga gga ata gat     2935
Ile Thr Glu Asn Met Ile Cys Ala Gly Tyr Glu Glu Gly Gly Ile Asp
950                 955                 960                 965

tct tgt cag ggg gat tca gga gga cca tta atg tgc caa gaa aac aac     2983
Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Met Cys Gln Glu Asn Asn
                970                 975                 980

agg tgg ttc ctt gct ggt gtg acc tca ttt gga tac aag tgt gcc ctg     3031
Arg Trp Phe Leu Ala Gly Val Thr Ser Phe Gly Tyr Lys Cys Ala Leu
            985                 990                 995

cct aat cgc  ccc gga gtg tat gcc  agg gtc tca agg ttt  acc gaa      3076
Pro Asn Arg  Pro Gly Val Tyr Ala  Arg Val Ser Arg Phe  Thr Glu
        1000                1005                1010

tgg ata caa  agt ttt cta cat tag cgcatttctt aaactaaaca ggaaagtcgc   3130
Trp Ile Gln  Ser Phe Leu His
        1015

attattttcc cattctactc tagaaagcat ggaaattaag tgtttcgtac aaaaatttta   3190
aaaagttacc aaaggttttt attcttacct atgtcaatga aatgctaggg ggccagggaa   3250
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cttaatgatt atttttatta cttactgttg tttaaaggga tgttatttta aagcatatac   3430
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aaccttagta ttttcccaac caatagaaac tatttattgt aagcttatgt cacaggcctg   3670
gactaaattg attttacgtt cctctt                                       3696
```

Fig. 6E

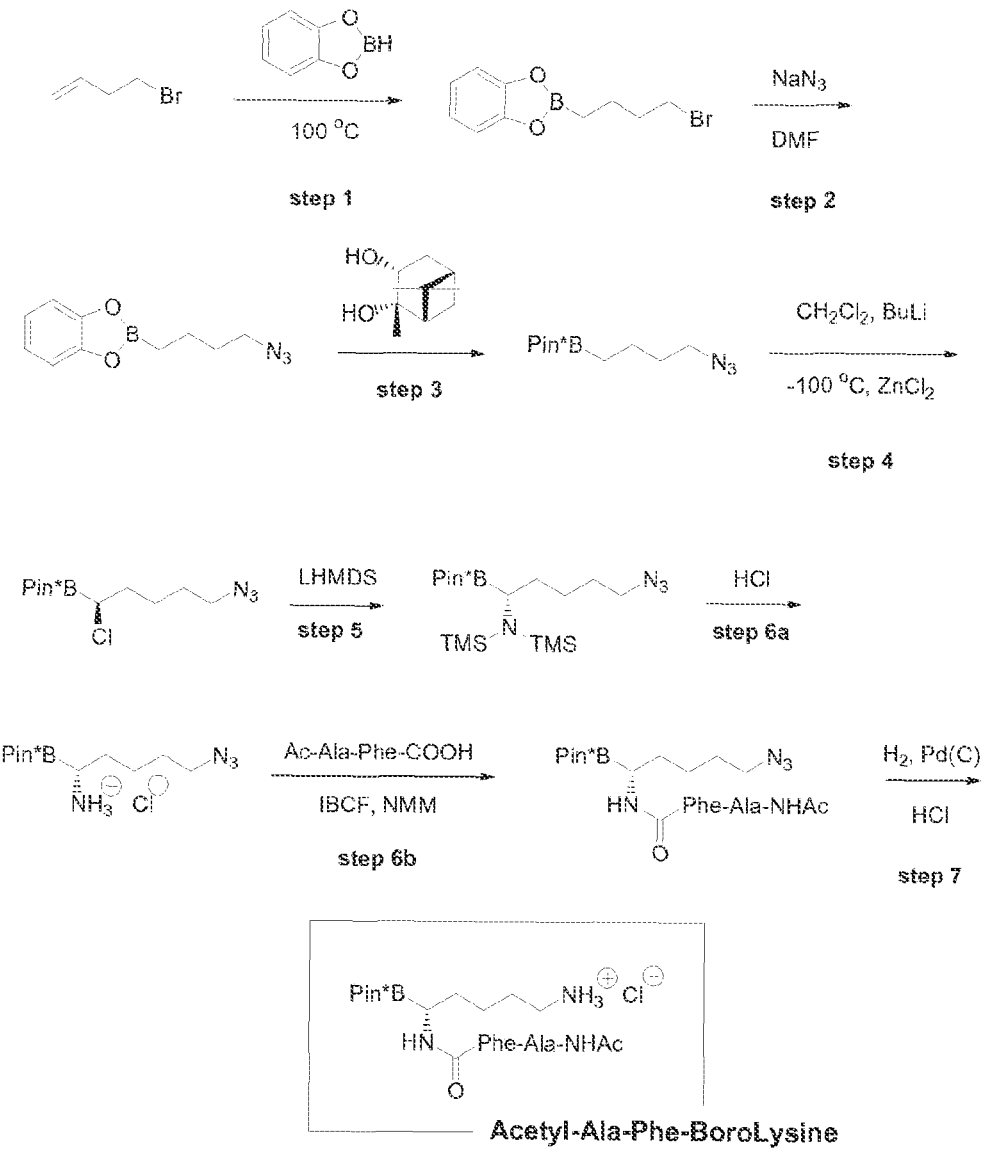
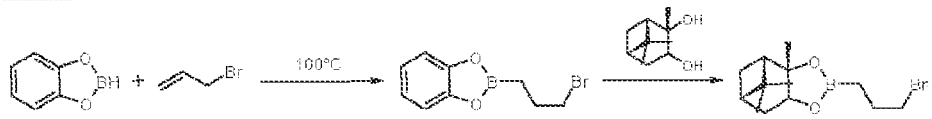

Br
BH
100 °C
step 1
NaN3
DMF
step 2
HO
HO
step 3
CH2Cl2, BuLi
-100 °C, ZnCl2
step 4
LHMDS
step 5
TMS
TMS
HCl
step 6a
Ac-Ala-Phe-COOH
IBCF, NMM
step 6b
H2, Pd(C)
HCl
step 7
Phe-Ala-NHAc
Acetyl-Ala-Phe-BoroLysine

Fig. 7A

Step 1:



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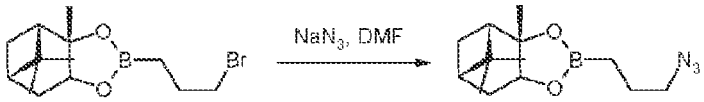

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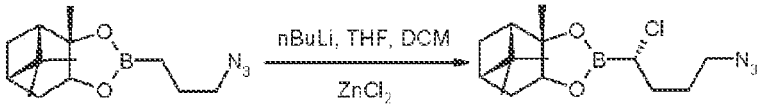

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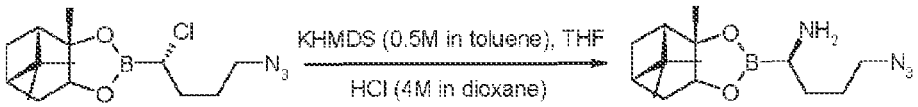

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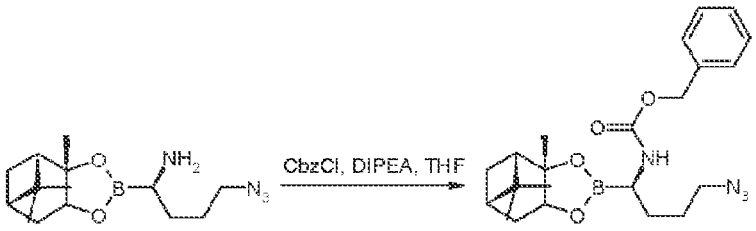

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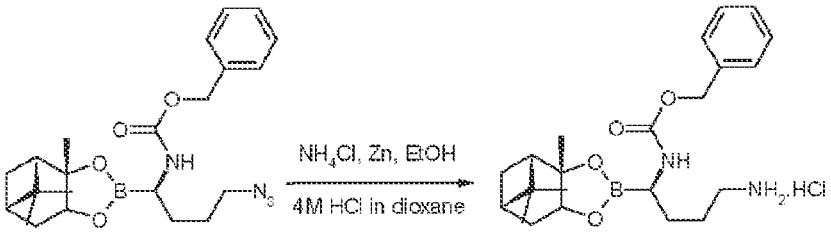
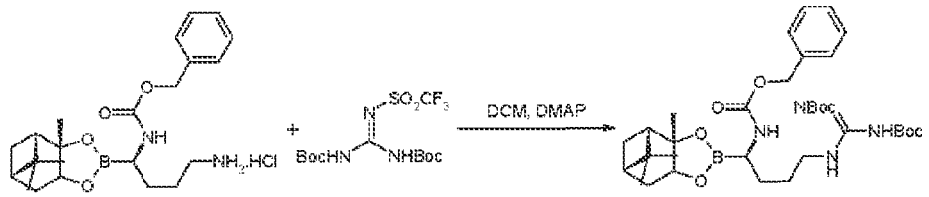


Step 7:

Fig. 7B



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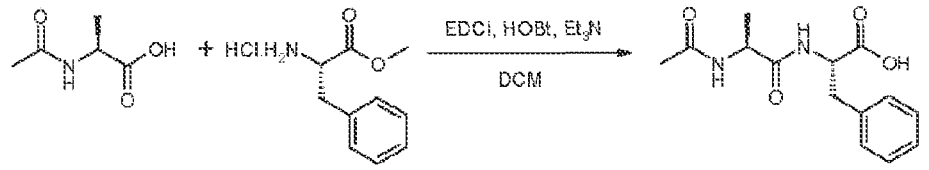

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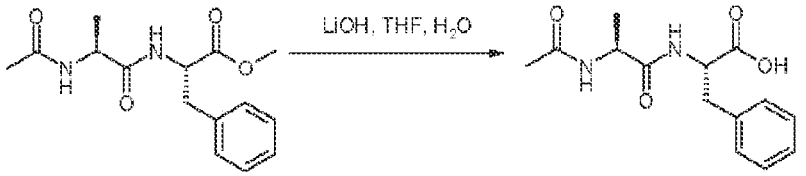

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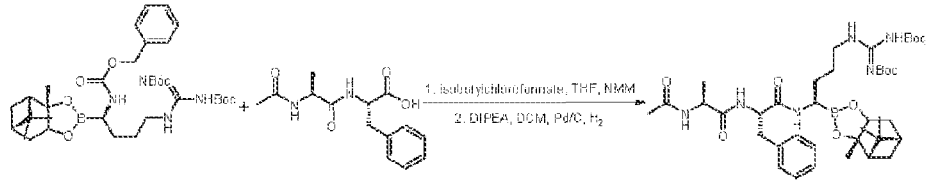

Step 11:

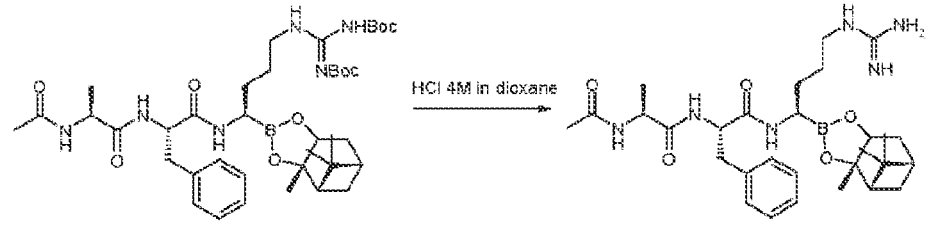

Fig. 7C

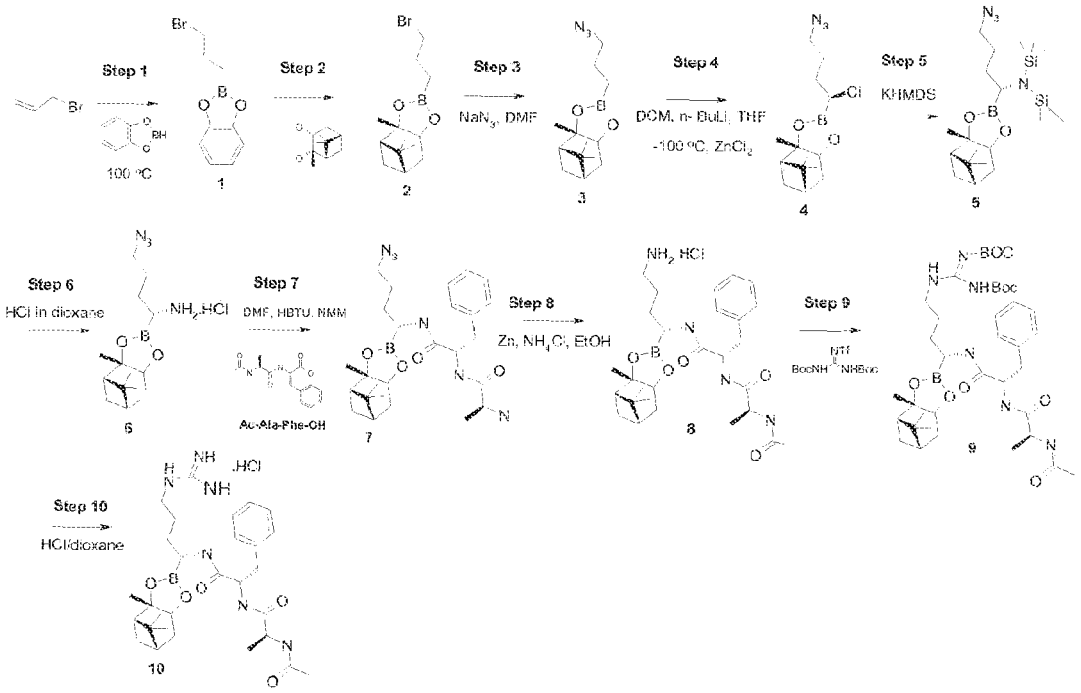
Step 1
100 °C
Step 2
Step 3
NaN3, DMF
Step 4
DCM, n-BuLi, THF
-100 °C, ZnCl2
Step 5
KHMDS
Step 6
HCl in dioxane
Step 7
DMF, HBTU, NMM
Ac-Ala-Phe-OH
Step 8
Zn, NH4Cl, EtOH
Step 9
Step 10
HCl/dioxane

Fig. 7D

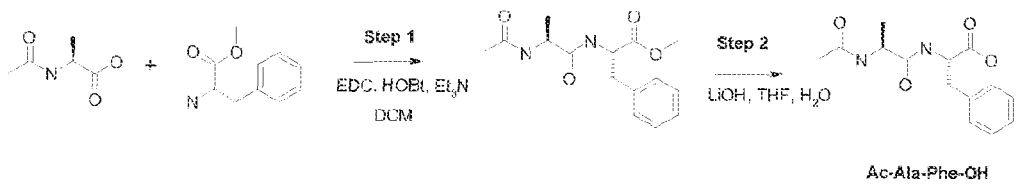
Step 1
EDC, HOBt, Et3N
DCM
Step 2
LiOH, THF, H2O
Ac-Ala-Phe-OH

Fig. 7E

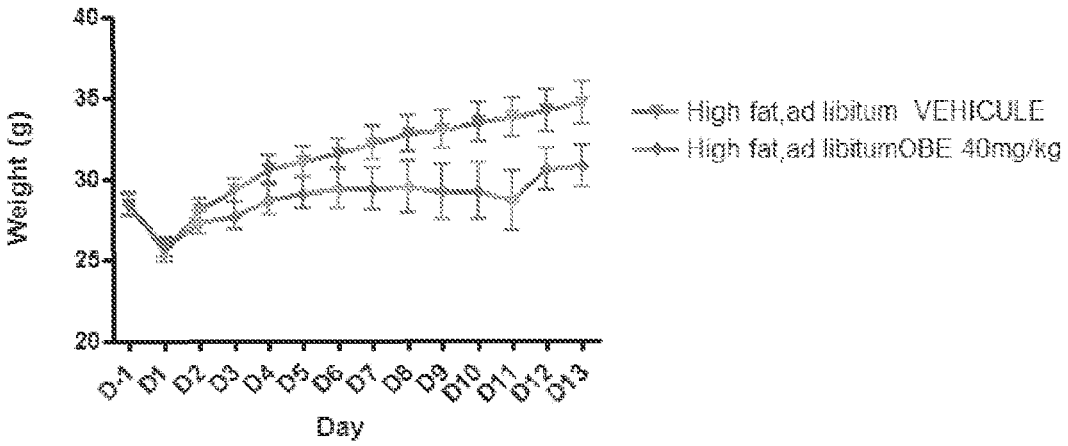
Weight (g)
40
35
30
25
20
D-1
D1
D2
D3
D4
D5
D6
D7
D8
D9
D10
D11
D12
D13
Day
High fat,ad libitum VEHICULE
High fat,ad libitumOBE 40mg/kg

Fig. 8

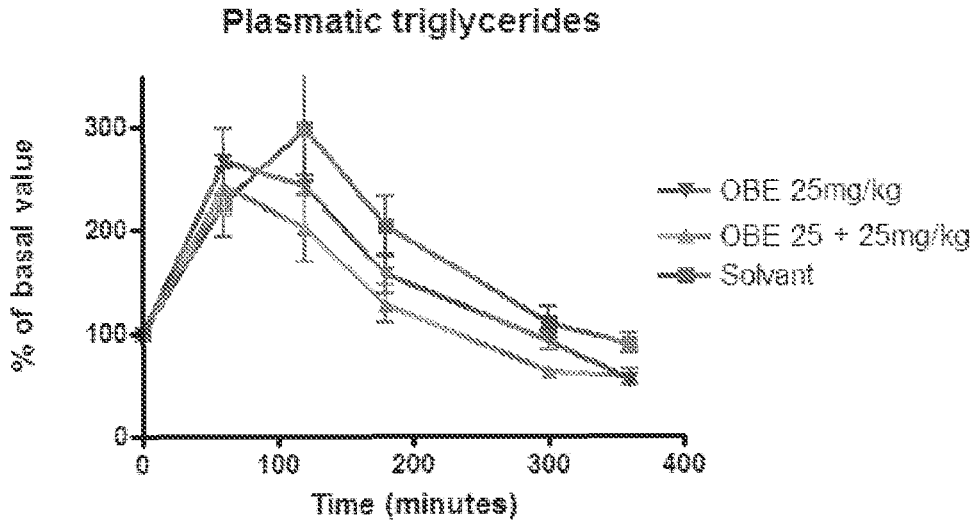
Plasmatic triglycerides
% of basal value
300
200
100
0
0
100
200
300
400
Time (minutes)
OBE 25mg/kg
OBE 25 + 25mg/kg
Solvant

Fig. 9

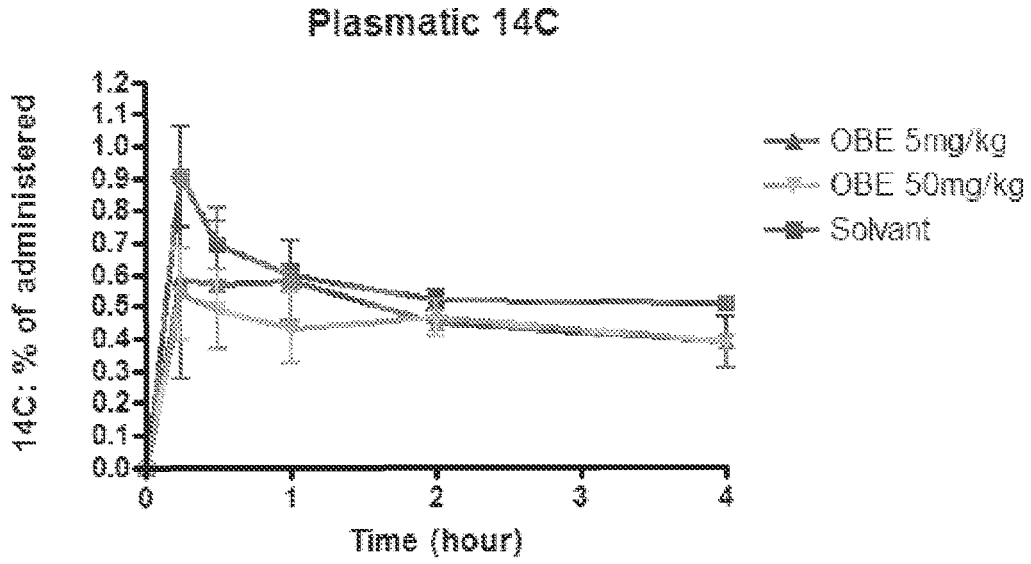
Plasmatic 14C
14C: % of administered
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
Time (hour)
OBE 5mg/kg
OBE 50mg/kg
Solvant

Fig. 10

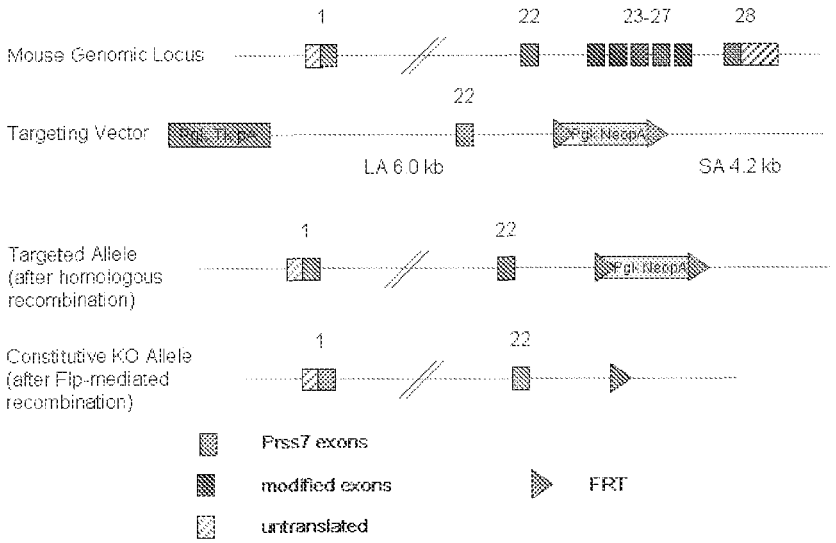

Fig. 11

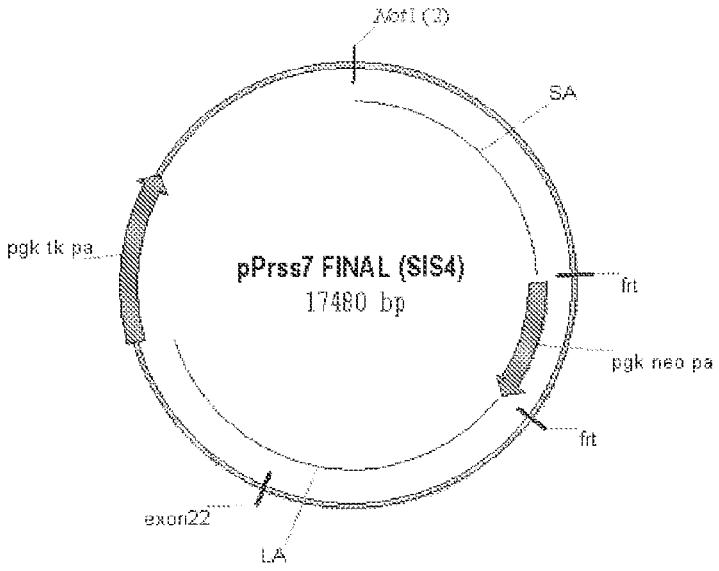

Fig. 12A

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Fig. 12B

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Fig. 12C

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Fig. 12D

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Fig. 12E

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Fig. 12F

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Fig. 12G

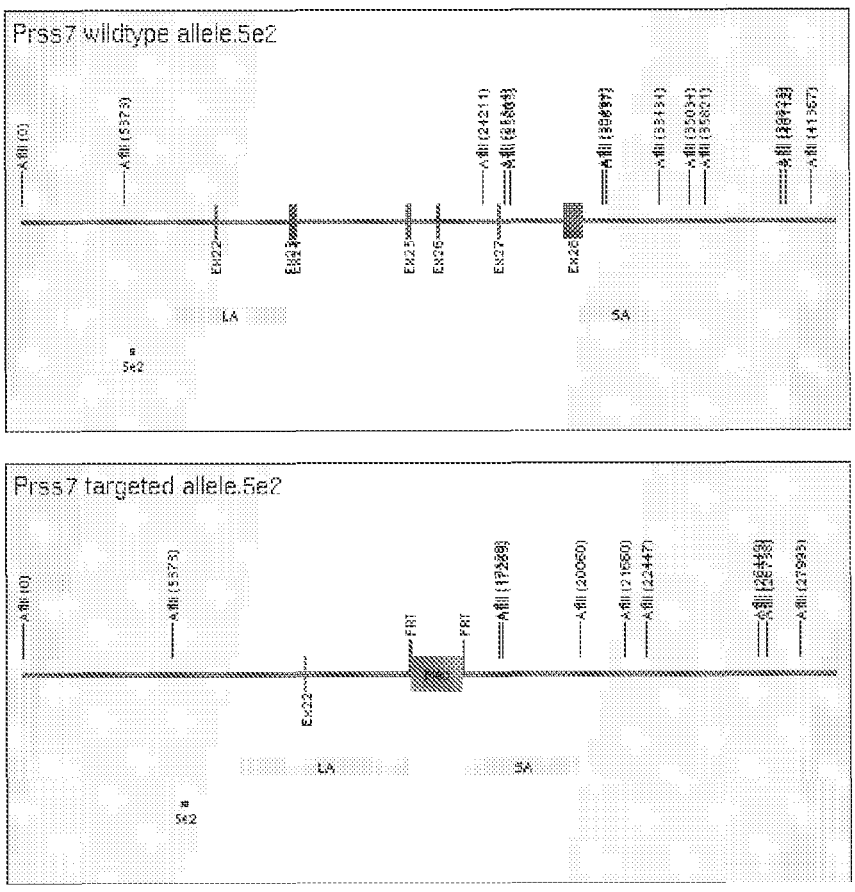


Fig. 13

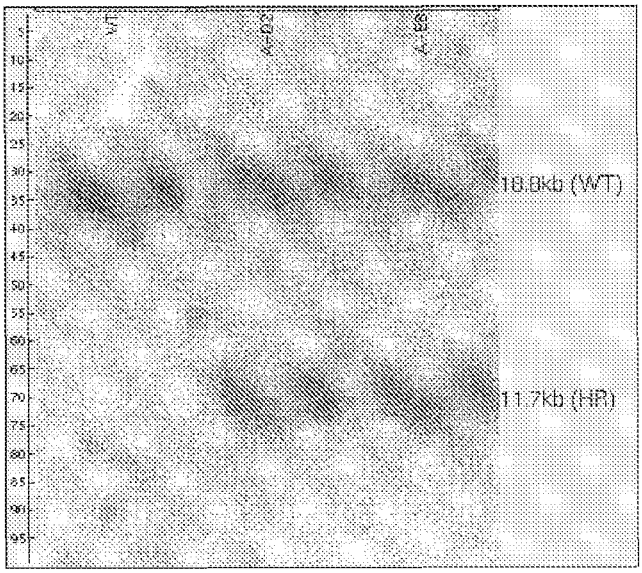

WT
A-03
A-05
10.8kb (WT)
11.7kb (HR)

Fig. 14

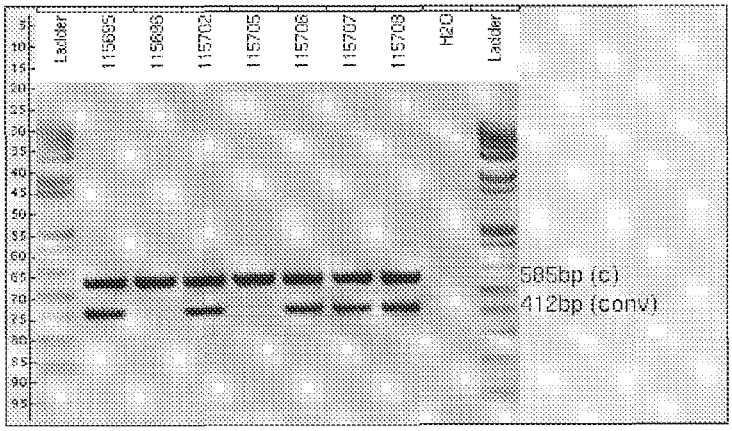

Ladder
115605
115606
115702
115705
115706
115707
115708
H2O
Ladder
585bp (c)
412bp (conv)

Fig. 15

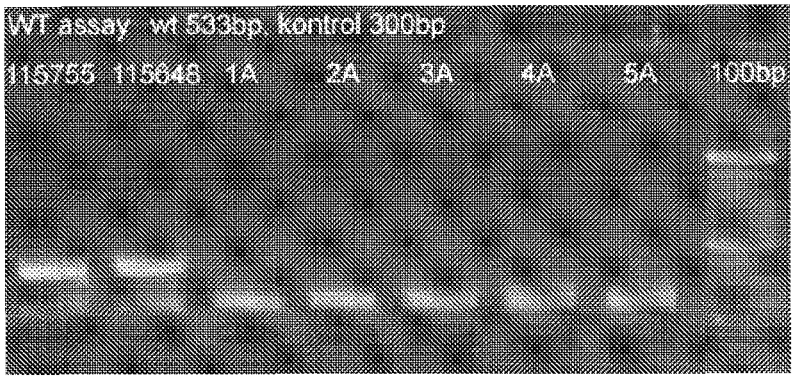

WT assay wt 533bp, kontrol 300bp
115755 115648 1A 2A 3A 4A 5A 100bp

Fig. 16

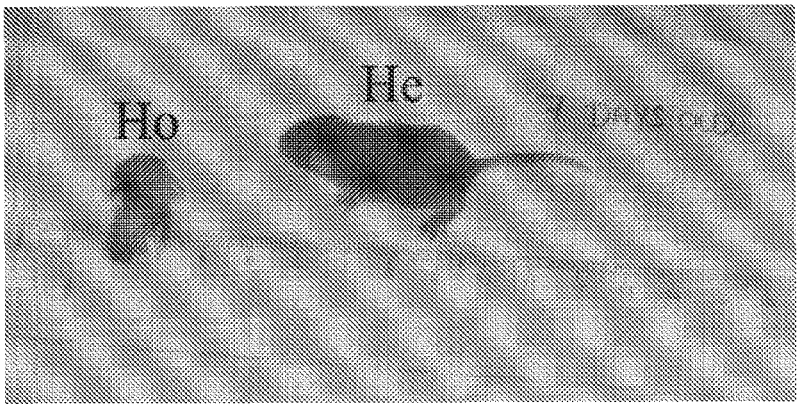

Ho
He

Fig. 17

```
accacc atg aat cca ctc ctg atc ctt acc ttt gtg gca gct gct ctt        48
       Met Asn Pro Leu Leu Ile Leu Thr Phe Val Ala Ala Ala Leu
       1               5                   10

gct gcc ccc ttt gat gat gat gac aag atc gtt ggg ggc tac aac tgt        96
Ala Ala Pro Phe Asp Asp Asp Asp Lys Ile Val Gly Gly Tyr Asn Cys
15                  20                  25                  30

gag gag aat tct gtc ccc tac cag gtg tcc ctg aat tct ggc tac cac       144
Glu Glu Asn Ser Val Pro Tyr Gln Val Ser Leu Asn Ser Gly Tyr His
                35                  40                  45

ttc tgt ggt ggc tcc ctc atc aac gaa cag tgg gtg gta tca gca ggc       192
Phe Cys Gly Gly Ser Leu Ile Asn Glu Gln Trp Val Val Ser Ala Gly
            50                  55                  60

cac tgc tac aag tcc cgc atc cag gtg aga ctg gga gag cac aac atc       240
His Cys Tyr Lys Ser Arg Ile Gln Val Arg Leu Gly Glu His Asn Ile
        65                  70                  75

gaa gtc ctg gag ggg aat gag cag ttc atc aat gca gcc aag atc atc       288
Glu Val Leu Glu Gly Asn Glu Gln Phe Ile Asn Ala Ala Lys Ile Ile
    80                  85                  90

cgc cac ccc caa tac gac agg aag act ctg aac aat gac atc atg tta       336
Arg His Pro Gln Tyr Asp Arg Lys Thr Leu Asn Asn Asp Ile Met Leu
95                  100                 105                 110

atc aag ctc tcc tca cgt gca gta atc aac gcc cgc gtg tcc acc atc       384
Ile Lys Leu Ser Ser Arg Ala Val Ile Asn Ala Arg Val Ser Thr Ile
                115                 120                 125

tct ctg ccc acc gcc cct cca gcc act ggc acg aag tgc ctc atc tct       432
Ser Leu Pro Thr Ala Pro Pro Ala Thr Gly Thr Lys Cys Leu Ile Ser
            130                 135                 140

ggc tgg ggc aac act gcg agc tct ggc gcc gac tac cca gac gag ctg       480
Gly Trp Gly Asn Thr Ala Ser Ser Gly Ala Asp Tyr Pro Asp Glu Leu
        145                 150                 155

cag tgc ctg gac gct cct gtg ctg agc cag gct aag tgt gaa gcc tcc       528
Gln Cys Leu Asp Ala Pro Val Leu Ser Gln Ala Lys Cys Glu Ala Ser
    160                 165                 170

tac cct gga aag att acc agc aac atg ttc tgt gtg ggc ttc ctt gag       576
Tyr Pro Gly Lys Ile Thr Ser Asn Met Phe Cys Val Gly Phe Leu Glu
175                 180                 185                 190

gga ggc aag gat tca tgt cag ggt gat tct ggt ggc cct gtg gtc tgc       624
Gly Gly Lys Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Val Val Cys
                195                 200                 205

aat gga cag ctc caa gga gtt gtc tcc tgg ggt gat ggc tgt gcc cag       672
Asn Gly Gln Leu Gln Gly Val Val Ser Trp Gly Asp Gly Cys Ala Gln
            210                 215                 220
```

Fig. 18A

```
aag aac aag cct gga gtc tac acc aag gtc tac aac tat gtg aaa tgg      720
Lys Asn Lys Pro Gly Val Tyr Thr Lys Val Tyr Asn Tyr Val Lys Trp
        225                 230                 235

att aag aac acc ata gct gcc aat agc taa agccccccagt atctcttcag      770
Ile Lys Asn Thr Ile Ala Ala Asn Ser
    240                 245

tctctatacc aataaagtga ccctgttctc                                   800
```

Fig. 18B

BOROPEPTIDE INHIBITORS OF ENTEROPEPTIDASE AND THEIR USES IN TREATMENT OF OBESITY, OVERWEIGHT AND/OR DISEASES ASSOCIATED WITH AN ABNORMAL FAT METABOLISM

FIELD OF THE INVENTION

The present invention relates to novel non-absorbable oligopeptides based on boroanalogs of amino acids incorporating a protonatable function on their side chain, such as boroarginine, borolysine, boroornithine, and related compounds, that selectively modulate, regulate, and/or inhibit enteropeptidase. These compounds are used individually, in combination or in association with other known compounds for the treatment of excess weight, obesity and diseases associated with an abnormal fat metabolism.

BACKGROUND AND RELATED PRIOR ART

Obesity is a multi-faceted chronic condition and is the most prevalent nutritional problem in the United States today. Obesity, a condition caused by an excess of energy intake as compared to energy expenditure, contributes to the pathogenesis of hypertension, type II or non-insulin dependent diabetes mellitus, hypercholesterolemia, hyperlipidemia, hypertriglyceridemia, heart disease, pancreatitis, and such common forms of cancer such as breast cancer, prostate cancer, uterine cancer and colon cancer.

At present, only a limited number of drugs for treating obesity are commercially available. Unfortunately, while some of these drugs may bring short-term relief to the patient, a long-term successful treatment has not yet been achieved. Exemplary methods of treating obesity are also disclosed in U.S. Pat. Nos. 3,867,539; 4,446,138; 4,588,724; 4,745,122; 5,019,594; 5,300,298; 5,403,851; 5,567,714; 5,573,774; 5,578,613; and 5,900,411.

One of the presently available drugs for treating obesity, developed by Hoffman-LaRoche, is an inhibitor of pancreatic lipase (PL). Pancreatic lipase is responsible for the degradation of triglycerides to monoglycerides. However, it has been associated with side-effects such as severe diarrhea resulting in absorption inhibition of only one specific fraction of fatty acids and, has been known to induce allergic reactions. Treatment with PL inhibitors is thus highly disadvantageous and may even expose the treated subject to life-threatening risks.

Recently, it has been suggested that fat absorption may be reduced by inhibiting the activity of the microsomal triglyceride-transfer protein (MTP), which is involved in the formation and secretion of very light density lipoproteins (VLDL) and chylomicrons. Sharp et al., [Nature (1993) 365:65-69] and Wetterau et al., [Science (1994) 282:751-754,] demonstrated that the mtp gene is responsible for abetalipoproteinemia disease. U.S. Pat. Nos. 6,066,650, 6,121,283 and 6,369,075 describe compositions that include MTP inhibitors, which are aimed at treating various conditions associated with excessive fat absorption. However, patients treated with MTP inhibitors suffer major side effects including hepatic steatosis, which are attributed to reduced MTP activity in both intestine and liver. This is not surprising since people naturally deficient for MTP activity were shown to develop fatty livers [Kane and Havel (1989); Disorders of the biogenesis and secretion of lipoproteins containing the apolipoprotein B. pp. 1139-1164 in: "The metabolic basis of inherited disease" (Scrivers et al., eds.). McGraw-Hill, New York]. In fact, the company Brystol

Myers Squibb, that developed MTP inhibitors for the treatment of obesity, has recently decided to abandon this target, due to this fatty liver side effect.

The presently known targets for the treatment of obesity and related disorders can be divided into four main classes: (i) appetite blockers, which include for example the NPY (neuropeptide Y); (ii) satiety stimulators, which include, for example, the product of the ob, db and agouti genes; (iii) energy or fatty acid burning agents, which include the UCPs (Uncoupling Proteins); and (iv) fat absorption inhibitors such as those acting on PL and MTP in the intestine, described above.

As discussed herein, the use of these targets is highly limited by their redundancy, their multiple targeting and/or their lack of tissue specificity.

There is thus a widely recognized need for, and it would be highly advantageous to have compositions and methods for treating obesity and related diseases and disorders devoid of the above limitations.

Serine proteases are involved in a large number of important physiological processes. Selective inhibition of a given serine protease is one of the strategies for the treatment of pathological conditions associated with the activity or overactivity of these serine proteases. Below is a non-exhaustive list of serine protease inhibitors disclosed in the literature:

- phosphorus-based inhibitors such as the diisopropylphosphofuloridate (DFP) (Jansen et al., (1952) Adv. Enzymol. 13: 321-343) or diphenyl phosphonate ester analogues;
- fluorine-containing serine proteases, such as trifluoromethyl ketones (TFMKs);
- peptide-based aldehydes, chloromethyl ketones, fluoromethyl ketones, dimethyl sulphonium salts, α-keto-acids and amides, α-keto esters and α-keto-aldehydes (glyoxals);
- natural products such as the cyclotheonamides, derived from the Japanese marine sponge *Theonella* sp.;
- molecules based on heterocyclic structure;
- N-hydroxysuccimide heteorcycles and related compounds;
- isocoumarins such as 3,4-dichloroisocoumarin;
- β lactam-based inhibitors;
- metal-potentiated compounds;
- aprotinin (Trasylol®), used to reduce bleeding; and
- serpins (serine protease inhibitors) such as antithrombin and α-1-antitrypsin having a role in coagulation/thrombosis and emphysema/A1AT respectively.

However, few compounds have been described as serine protease inhibitors with a specific and selective inhibition of a unique target. Moreover, no compounds have been disclosed or suggested, to selectively and specifically inhibit the enteropeptidase, and to be used in the treatment of obesity, excess weight or diseases associated with an abnormal fat metabolism.

Enteropeptidase is a serine protease situated on the surface of epithelial intestinal cells (enterocytes) (Lancet. 1969 Apr. 19; 1(7599):812-3; Am J Physiol Gastrointest Liver Physiol. 2003 December; 285(6):G1235-41; Proc Soc Exp Biol Med. 1994 June; 206(2):114-8; Ciba Found Symp. 1979 Jan. 16-18; (70):169-87; Lancet. 1982 Aug. 28; 2(8296):504). The substrate of enteropeptidase is trypsinogen, a precursor to trypsin. Enteropeptidase converts trypsinogen into a molecule of trypsin. In turn, trypsin, which is also a serine protease, converts the precursors of a series of digestive enzymes, such as procarboxypeptidases A and B, chymotrypsinogen, pancreatic prolipase and proelastase, into the active forms of the enzymes (carboxypep-

tidases A and B, chymotrypsin, pancreatic lipase and elastase). The latter active forms of such digestive enzymes are required for the processing and ultimate absorption of protein and fat matter in the gastrointestinal (GI) tract.

Because enteropeptidase is located in the intestinal lumen, inhibition of this enzyme requires that the compounds selectively inhibit enteropeptidase without interfering with circulating serine proteases, such as thrombin, kalikrein, and the like.

Thus, there is a need for compounds to treat obesity, excess overweight as well as diseases associated with an abnormal fat metabolism, on a long term basis that have a specific target.

It is an object of the present invention to provide compounds that inhibit enteropeptidase, and more particular that selectively inhibit enteropeptidase. In particular, these compounds are non-absorbable i.e., they do not pass from the intestine into the blood.

It is another object of the present invention to provide compounds that are derivatives of boroanalogs of amino acids incorporating a protonatable functionality on their side chain, such as borolysine, boroornithine, boroarginine and the like, and are strong, non-absorbable inhibitors of enteropeptidase.

Yet another object of the invention, are compositions, especially pharmaceutical compositions, comprising at least one of the compounds disclosed in the present invention.

It is also another object of the invention to provide methods to treat obesity, excess weight or diseases associated with an abnormal fat metabolism, comprising administrating, to a mammal in need thereof, at least one of the compounds disclosed in the present invention or a composition described in the present invention.

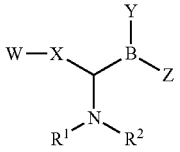
Yet another object of the present invention is the use of at least one of the compounds or of the composition disclosed herein, for the treatment of obesity, excess weight and diseases associated with an abnormal fat metabolism. A compound or a composition of the invention for use in the treatment of obesity, excess weight and diseases associated with an abnormal fat metabolism, is also provided.

These and other objects are achieved by the present invention as evidence by the summary of the invention, description of the preferred embodiments and the claims.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to a compound having the following formula (I):

I

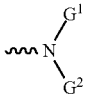


wherein

B represents a boron atom;

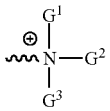
W is a nitrogen-containing functionality group, sustaining a positive charge either through protonation or quaternization, this group being selected from:

(i) an amino group of the formula:



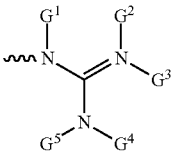
wherein G^1 and G^2 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates the N atom and that contains a total of up to 15 atoms;

(ii) a quaternary ammonium group of formula:



wherein G^1, G^2 and G^3 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates the N atom and that contains a total of up to 15 atoms; or

(iii) a guanidine group of formula:



wherein G^1, G^2, G^3, G^4 and G^5 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates one or more of the guanidine N atoms and that contains a total of up to 15 atoms;

X is a linker unit having the formula $(CX^1X^2)_p$, wherein $1<p<10$, and wherein X^1 and X^2 are, independently, H, or linear or branched alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring residue containing from 3 to 10 atoms;

Y is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms;

Z is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms, or is a linear or branched or cyclic alkyl group containing from 1 to 15 carbon atoms;

R1 is chosen among:

(i) an aminoacyl residue derived from an amino acid.

(ii) a derivative of the foregoing aminoacyl residue in which the amino group is additionally acylated, or sulfonylated, or phosphorylated to form an amide, or peptide, or sulfonamide, or phosphoramide bond;

(iii) an acyl group of general formula R'—CO, wherein R' is:

a. a linear, branched or cyclic alkyl group that contains from 1 to 10 C atoms;

b. a saturated heterocyclic ring incorporating up to 20 atoms chosen from C, O, N, and S atoms;

c. an aryl group selected from phenyl or a substituted variant thereof bearing any combination, at any one ring position, of one or more substituents

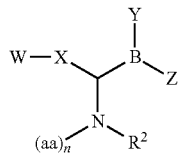
selected from halogen, alkyl groups containing from 1 to 10 carbon atoms, trifluoromethyl and alkoxy groups; or

d. a heteroaryl group;

R^2 is either H, or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms, or an OR group wherein R may be H or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms.

In another aspect, the invention also concerns a compound having the formula II.

II

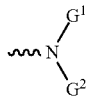


wherein

B represents a boron atom;

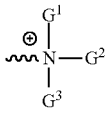
W is a nitrogen-containing functionality group, sustaining a positive charge either through protonation or quaternization, this group being selected from:

(i) an amino group of the formula:



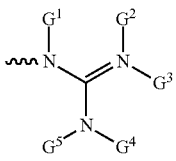
wherein G^1 and G^2 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates the N atom and that contains a total of up to 15 atoms;

(ii) a quaternary ammonium group of formula:



wherein G^1, G^2 and G^3 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates the N atom and that contains a total of up to 15 atoms; or

(iii) a guanidine group of formula:



wherein G^1, G^2, G^3, G^4 and G^5 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates one or more of the guanidine N atoms and that contains a total of up to 15 atoms;

X is a linker unit having the formula $(CX^1X^2)_p$, wherein $1<p<10$, and wherein X^1 and X^2 are, independently, H, or linear or branched alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring residue containing from 3 to 10 atoms;

Y is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms;

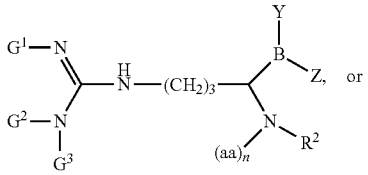
Z is an OH, or an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms, or is a linear or branched or cyclic alkyl group containing from 1 to 15 carbon atoms;

R^2 is either H, or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms; or an OR group, wherein R may be H or a or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms; and

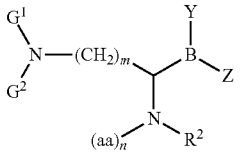
"aa" refers to any amino acyl residue or derivative thereof, and n is at least two.

In a yet another aspect, the invention is directed to the following compounds having formula (III) or (IV):

III



IV



wherein:

B represents a boron atom;

Y is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms;

Z is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms, or is a linear or branched or cyclic alkyl group containing from 1 to 15 carbon atoms;

R^2 is either H, or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms or an OR group wherein R may be H or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms;

G^1, G^2 and G^3 or G^1 and G^2 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates one or more of the guanidine N atoms and that contains a total of up to 15 atoms;

"aa" refers to any amino acid residue or derivative thereof, and n is at least two; and

the index, m, equals 3 or 4.

In another aspect, the invention also concerns a pharmaceutical composition comprising at least one compound as described above.

In a last aspect, the invention also refers to a method comprising administering to a mammal in need of said treatment, at least one compound or a composition of the invention for treating a patient having obesity, having excess weight or suffering from abnormal fat metabolism.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a graph showing the activity of the enteropeptidase according to increasing concentrations of compound OBE 1999.

FIG. **2** is a graph showing the activity of the enteropeptidase according to increasing concentrations of compound OBE 2000.

FIG. **3** is a graph showing the activity of the enteropeptidase according to increasing concentrations of compound OBE 2001.

FIG. **4** is a graph showing the activity of the enteropeptidase according to increasing concentrations of compound OBE 2002.

FIG. **5** is a Table showing the IC 50 (in nM) of enteropeptidase, various serine proteases and other enzyme families, in the presence of compounds OBE 1999, OBE 2000, OBE 2001 or OBE 2002.

FIGS. **6**A-**6**E represent the nucleotide and protein sequences of the human enteropeptidase (PRSS7). The first line indicates the nucleotide sequence, grouped by codons; the second line indicates the amino acid sequence corresponding to the above codons with the three-letter code. The first codon of translation and the stop codon are shown in bold. Numbering of the nucleic acid is at the right end of the first line, and numbering of the amino acids is indicated under amino acid residue (third line).

FIGS. **7**A-E represent examples of processes to synthesize the compounds of the invention. (FIG. **7**A): example of a process for the synthesis of the Acetyl-Ala-Phe-BoroLysine (*pin: pinandiol group); (FIG. **7**B and FIG. **7**C): first example of a process for the synthesis of the Acetyl-Ala-Phe-BoroArginine; (FIG. **7**D): second example of a process for the synthesis of the Acetyl-Ala-Phe-BoroArginine; this second process also enables the synthesis of the Acetyl-Ala-Phe-BoroOrnithine (compound 8), since Acetyl-Ala-Phe-BoroOrnithine is an intermediate compound in the synthesis of Acetyl-Ala-Phe-BoroArginine by this process; (FIG. **7**E): synthesis of the Acetyl-Ala-Phe-OH group, to be inserted in step 10 of the process in FIG. **7**C or in step 7 of the process in FIG. **7**D.

FIG. **8** is a graph illustrating the follow up in days of the weight (in grams) of mice having received water (vehicle) as compared to mice having received OBE2001 at a concentration of 40 mg/kg/day (B).

FIG. **9** is a graph illustrating the follow up in minutes of the triglycerides present in the plasma of mice having received water (solvent), one dosage of OBE2001 (OBE 25 mg/kg) or two dosages of OBE2001 (OBE 25+25 mg/kg).

FIG. **10** is a graph illustrating the follow up in hours of labelled proteins in plasma of mice having received water (solvent), one dosage of OBE2001 (OBE 5 mg/kg) or two dosages of OBE2001 (OBE 50 mg/kg).

FIG. **11** is a schematic representation of the process used to obtain a constitutive enteropeptidase knockout (KO) allele in mice, using the deletion of the exons 23-28 of the Prss7 gene.

FIG. **12**A is a schematic representation of a vector comprising the constitutive enteropeptidase knockout (KO) allele. FIGS. **12**B-G represent the 17,480 bp nucleotide sequence of this vector.

FIG. **13** is the Afill restriction map of the wild type enteropeptidase allele (above) and the knockout (KO) enteropeptidase allele (below).

FIG. **14** is a Southern Blot obtained with genomic DNA from embryonic stem (ES) cells successfully transformed with the enteropeptidase KO allele (A-D2 and A-E8) and obtained with the genomic DNA of non transformed cells (WT).

FIG. **15** is a blot in which PCR fragments have been run, from a simultaneous amplification with primers 1260_1 and 1260_2, and 1472_23 and 1472_24. Genomic DNA has been extracted from heterologous mice (115695, 115702, 115706, 115707 and 115708) or wild type mice (115696 or 115705). 585 bp (c): control fragment; 412 bp (cony): KO allele.

FIG. **16** is a blot in which PCR fragments, obtained from a simultaneous amplification with primer 1472_23 and primer PRRS7 WT, and beta actin forward and beta actin backward primers, have been run. Genomic DNA has been extracted from heterologous mice (115755 and 115648) or KO homologous pups (1A, 2A, 3A, 4A and 5A); 533 bp (c): WT enteropeptidase allele; 300 bp: control allele (actin).

FIG. **17** is a photograph showing a mouse homozygous for the enteropeptidase knockout allele (Ho) and a mouse heterozygous for the enteropeptidase knockout allele (He), both 7 days old.

FIGS. **18**A-B represent the nucleotide and protein sequences of human trypsin.

DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

As used herein the term "protecting group" means a chemical group used to modify the compound of the invention, in one of its functional groups, in order to obtain in a subsequent chemical reaction and to avoid unwanted reactions. Examples of protecting groups are as follows:

- Alcohol protecting groups: Acetyl (Ac), β-Methoxyethoxymethyl ether (MEM), Methoxymethyl ether (MOM), p-Methoxybenzyl ether (PMB), Methylthiomethyl ether, Pivaloyl (Piv), Tetrahydropyran (THP), Silyl ether (most popular ones include trimethylsilyl (TMS), tert-butyldimethylsilyl (TBDMS), and triisopropylsilyl (TIPS) ethers) and methyl ethers;
- Amine protecting groups: Acetyl (Ac), Benzoyl (Bz), Pivaloyl (Pv) Methanesulfonyl (Ms), Benzenesulfonyl ($PhSO_2$), para-Toluenesulfonyl (Ts), Phosphoryl [$(HO)_2P(O)$], Dibenzyloxyphosphoryl [$(BnO)_2P(O)$], Benzyloxy-methanephosphonyl [$(CH_3)(BnO)P(O)$], Carbobenzyloxy (Cbz) group, tert-Butyloxycarbonyl (BOC) group, 9-Fluorenylmethyloxycarbonyl (FMOC) group, Benzyl (Bn) group and p-methoxyphenyl (PMP) group;
- Carbonyl protecting groups: Acetals and Ketals, Acylals and Dithianes; and

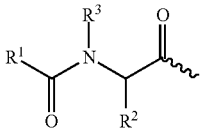
Carboxylic acid protecting groups: Methyl esters, Benzyl esters, tert-Butyl esters and Silyl esters.

"Chemoselectivity" as used herein means the preferential outcome of one instance of a generalized reaction over a set of other plausible reactions.

"An amino acid residue" is defined in the present invention as one of the following 21 amino acid acyl residues: glycine, alanine, valine, leucine, isoleucin, phenylalanine, tyrosine, tryptophan, methionine, cystine, cysteine, serine, threonine, histidine, aspartic acid, glutamic acid, asparagine, glutamine, lysine, arginine and proline.

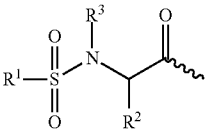
A "derivative of an amino acid residue" means an amino acid residue as defined in the previous paragraph, for which the amino group is additionally acylated, or sulfonylated, or phosphorylated to form an amide, or carbamate, or urea, or N-substituted urea, or peptide, or sulfonamide, or phosphoramide bond. Additionally, the amino group may be further substituted with a small alkyl group containing from 1 to 5 C atoms. Particular derivatives of amino acids are exemplified by, but not limited to, the following representative structures, (i)-(iii), wherein the wavy line represents the bond that connects these structures to the other part of the molecule:

structural type (i)



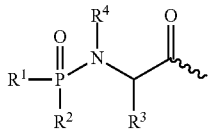
R^1	R^2	R^3	name
CH_3	CH_3	H	N-acetylalanyl
CH_3	CH_3	CH_3	N-methyl-N-acetylalanyl
CH_3	$(CH_2)_2COOH$	H	N-acetylglutamyl
CH_3	CH—C_6H_5	H	N-acetylphenylalanyl
$(CH_3)_3C$	CH_3	CH_3	N-methyl-N-pivaloylalanyl
C_6H_5	$(CH_2)COOH$	H	N-benzoylglutamyl
4-Cl—C_6H_4	$(CH_2)COOH$	H	N-(4-chlorobenzoyl)glutamyl
$OCH_2C_6H_5$	CH_3	H	N-(carbobenzyloxy)alanyl
NH_2	CH—C_6H_5	H	N-(carbamoyl)phenylalanyl
$N(CH_2CH_3)_2$	CH_3	H	N-(diethylcarbamoyl)alanyl

structural type (ii)



R^1	R^2	R^3	name
CH_3	CH_3	H	N-methanesulfonylalanyl
CH_3	CH_3	CH_3	N-methyl-N-methanesulfonylalanyl
4-CH_3—C_6H_4	$(CH_2)_2COOH$	H	N-p-toluenesulfonylglutamyl
CH_3	CH—C_6H_5	H	N-methansulfonylphenylalanyl
C_6H_5	CH_3	CH_3	N-methyl-N-benzenesulfonylalanyl

structural type (iii)



R^1	R^2	R^3	R^4	name
OH	OH	CH_3	H	N-phosphorylalanyl
$OCH_2C_6H_4$	$OCH_2C_6H_4$	CH_3	H	N-(dibenzyloxyphosphoryl)alanyl
$OCH_2C_6H_4$	$OCH_2C_6H_4$	CH_3	CH_3	N-methyl-N-(dibenzyloxyphosphoryl)alanyl
CH_3	OH	CH_3	H	N-(methanephosphonyl)alanyl

The expression "pharmaceutically acceptable salt" means an acid salt or a basic salt that is suitable or compatible with the treatment of the subject.

By "inhibition of the activity of the enteropeptidase", is meant that a decrease of 50% of the in vitro activity of the enteropeptidase is obtained, with a concentration of the compound of the invention that is less than 10 μM, less than 1 μM, less than 100 nM, less than 10 nM or less than 1 nM, for 1 nM of enteropeptidase. Said concentration can be determined as described in details in point 2.1. below, and particularly using Np Tosyl Gly Pro Arg pNa as a substrate.

The expression "specific inhibition of the trypsin-like serine protease" refers to the inhibition of proteases from the serine protease family and more particularly to serine protease of the trypsin-like subtype only. In contrast, the activity of other proteases such as cysteine peptidases, aspartate peptidases, metallo-proteases, lipases and/or glucosidases are not altered by the compound of the invention. In another embodiment, in combination with the previous one, the activity of chymotrypsin-like serine proteases is not altered by compound of the invention.

The expression "selective inhibition of the enteropeptidase" refers to the inhibition of the enteropeptidase only (in vivo), whereas the activity of other proteases from the same subtype (trypsin-like subtype) are not altered by the compounds of the invention. This distinction between the specificity and selectivity has been rendered possible by the fact that the compounds of the invention are non-absorbable and therefore the inhibition is limited to the enteropeptidase whose location is intestinal.

The determination of the IC_{50} of the compounds of the invention on enteropeptidase or on other serine protease may be tested in vitro as described in the examples below.

The term "treatment" as used herein refers not only to the loss of weight of the mammal following the administration of at least one compound or the composition of the invention, but also to the maintenance of the weight such that there is no weight increase.

The term "mammal" encompasses any of various warm-blooded vertebrate animals of the class Mammalia, including humans, characterized by a covering of hair on the skin and, in the female, milk-producing mammary glands for nourishing the young.

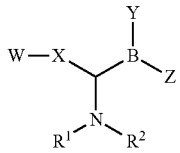
"Pharmaceutically acceptable vehicles or carriers" encompass any substance that enables the formulation of the compounds of the invention within a composition. A vehicle is any substance or combination of substance physiologically acceptable i.e., appropriate for its use in a composition in contact with a mammal, and thus non-toxic. Examples of such vehicles are phosphate buffered saline solutions, distilled water, emulsions such as an oil/water emulsions, various types of wetting agents sterile solutions and the like.

"Obesity" is in general defined in human by at least 20% over the average weight for the person's age, sex and height. Obesity is defined by a body mass index (BMI=kg/m^2) over 30. Obesity can also be defined by absolute waist circumference (>102 cm in men and >88 cm in women) or Waist-to-hip ratio (WHR) (WHR more than 0.7 for women and more than 0.9 for men). "Excessive weight" is defined by a BMI that is comprised between 25 and 29.9.

More specifically, in a first embodiment, the present invention relates to compounds having an inhibitory activity on the enzymatic activity of enteropeptidase. In another aspect, compounds are provided that have a selective inhibitory activity on the enzymatic activity of enteropeptidase.

The compounds of the invention have the following formula I:

I



wherein:

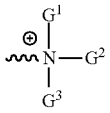
B represents a boron atom;

W is a nitrogen-containing functionality group, sustaining a positive charge either through protonation or quaternization, this group being selected from:

(i) an amino group having the formula:

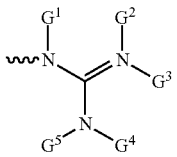
wherein G^1 and G^2 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates the N atom and that contains a total of up to 15 atoms.

(ii) a quaternary ammonium group having the formula:



wherein G^1, G^2 and G^3 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates the N atom and that contains a total of up to 15 atoms;

(iii) a guanidine group having the formula:



wherein G^1, G^2, G^3, G^4 and G^5 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates one or more of the guanidine N atoms and that contains a total of up to 15 atoms;

X is a linker unit having the formula $(CX^1X^2)_p$, wherein $1<p<10$, and wherein X^1 and X^2 are, independently, H, or linear or branched alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring residue containing from 3 to 10 atoms.

Y is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms;

Z is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms, or is a linear or branched or cyclic alkyl group containing from 1 to 15 carbon atoms;

R1 is selected from:

(i) an aminoacyl residue derived from an amino acid.

(ii) a derivative of the foregoing aminoacyl residue in which the amino group is additionally acylated, or sulfonylated, or phosphorylated to form an amide, or peptide, or sulfonamide, or phosphoramide bond;

(iii) an acyl group of general formula R'—CO, wherein R' is:

a. a linear, branched or cyclic alkyl group that contains from 1 to 10 C atoms;

b. a saturated heterocyclic ring incorporating up to 20 atoms chosen from C, O, N, and S atoms;

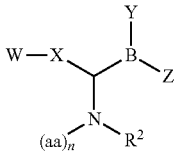
c. an aryl group selected from phenyl or a substituted variant thereof bearing any combination, at any one ring position, of one or more substituents selected from halogen, alkyl groups containing from 1 to 10 carbon atoms, trifluoromethyl and alkoxy groups; or

d. a heteroaryl group.

For compounds of formula (I) above, examples of a heteroaryl group (R^1) is a 2,3, or 4-pyridyl group, which may additionally bear any combination of one or more substituents such as halogen, alkyl groups containing from 1 to 10 carbon atoms, trifluoromethyl, alkoxy, alkylthio, alkylsulfonyl, etc. Similarly, examples of a five-membered ring aromatic heterocyclic group (R^1) are 2-thienyl, 3-thienyl, 2-thiazolyl, 4-thiazolyl and 5-thiazolyl groups. These groups may additionally bear any combination of one or more substituents such as halogen, an alkyl group containing from 1 to 10 carbon atoms, trifluoromethyl, alkoxy, alkylthio, alkylsulfonyl, etc.

In another embodiment, the present invention relates to the compound having the formula II:

II

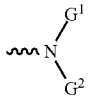


wherein

B represents a boron atom;

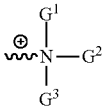
W is a nitrogen-containing functionality group, sustaining a positive charge either through protonation or quaternization, this group being selected from:

(i) an amino group having the formula:



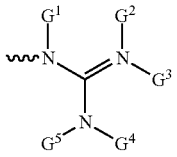
wherein G^1 and G^2 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates the N atom and that contains a total of up to 15 atoms;

(ii) a quaternary ammonium group having the formula:



wherein G^1, G^2 and G^3 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates the N atom and that contains a total of up to 15 atoms;

(iii) a guanidine group having the formula:



wherein G^1, G^2, G^3, G^4 and G^5 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates one or more of the guanidine N atoms and that contains a total of up to 15 atoms;

X is a linker unit having the formula $(CX^1X^2)_p$, wherein $1<p<10$, and wherein X^1 and X^2 are, independently, H, or linear or branched alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring residue containing from 3 to 10 atoms;

Y is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms;

Z is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms, or is a linear or branched or cyclic alkyl group containing from 1 to 15 carbon atoms;

R^2 is either H, or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms, or an OR group wherein R may be H or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms; and

"aa" refers to any amino acyl residue or derivative thereof, and n is at least two, or in another embodiment n is between 2 and 20, in another embodiment between 2 and 15, in another embodiment between 2 and 10, and in another embodiment between 2 and 5.

With respect to the compounds of formula (II), in another aspect, n is 2, 3 or 4. The at least two amino acid residues are selected among the following: Ala, Arg, Asn, Asp, Cys, Cystine, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val.

In the case where n=2, a combination of amino acid residues is Tyr and Pro, Ala and Phe, Arg and Glu, Glu and Gly, Trp and Glu, Thr and Pro, and Leu and Leu.

In the case where n=3, a combination of amino acid residues is Phe, Arg and Val, Arg, Cys and Thr, Gly, Cys and Pro, Gly, Cys and Asn and Lys, Gly and Asp.

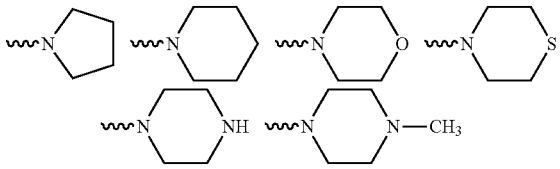
In yet another embodiment, the amino group of the first N-terminal amino acid residue bears a protecting group selected from the group of acyl, sulfonyl, or phosphoryl, as specified on page 10, and it may further bear an alkyl group containing from 1 to 5 C atoms, as indicated in the table that appears on page 11.

In yet another embodiment, the amino group of the first N-terminal amino acid residue can be derivatized with an acyl, sulfonyl, or phosphoryl group to form an amide, or carbamate, or urea, or N-substituted urea, or peptide, or sulfonamide, or phosphoramide bond, and it may be further substituted with a small alkyl group containing from 1 to 5 C atoms, as indicated in the table that appears on page 11.

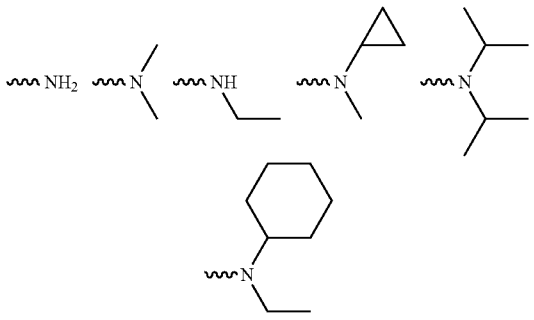
With respect to the compounds of formula (I) or (II) as defined above:

When G^1 to G^5 are, independently, branches of a ring system that incorporates the N atom and that contains a total of up to 15 atoms, these 15 atoms include any permutation of carbon, oxygen, nitrogen, and sulfur atoms,

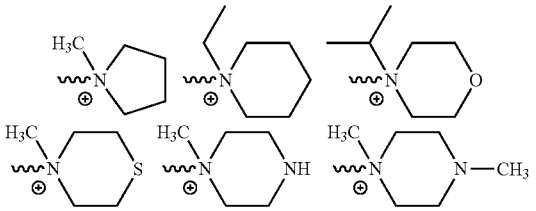
Examples of ring residues for the amino group (W) are pyrrolidine, piperidine, morpholine, thiomorpholine, piperazine, and N-methylpiperazine. Representative embodiments are as follows:



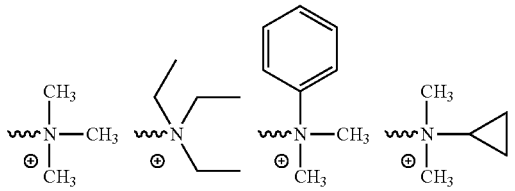
Examples of G^1 and G^2 for the amino group (W) are the following: hydrogen, methyl, ethyl, propyl, isopropyl, cyclopropyl, butyl, isobutyl, tert-butyl, cyclobutyl, pentyl, cyclopentyl, hexyl, cyclohexyl, phenyl, etc. Representative embodiments are as follows:



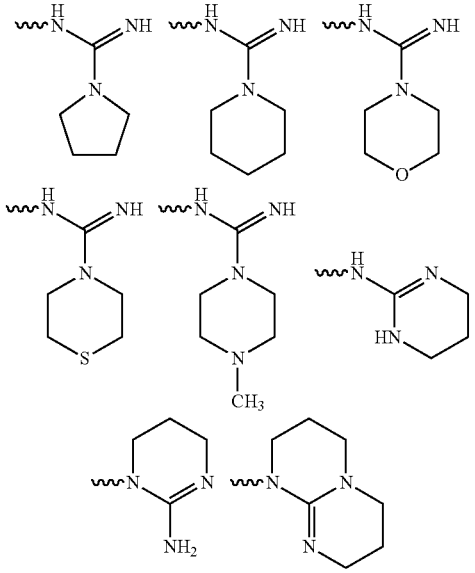
Examples of ring residues for the quaternary ammonium group (W) are pyrrolidine, piperidine, morpholine, thiomorpholine, piperazine, and N-methylpiperazine. Representative embodiments are as follows:



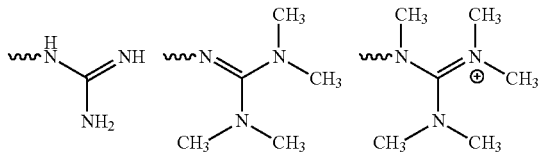
Examples of G^1, G^2 and G^3 for the quaternary ammonium group (W) are the following: methyl, ethyl, propyl, isopropyl, cyclopropyl, butyl, isobutyl, tert-butyl, cyclobutyl, pentyl, cyclopentyl, hexyl, cyclohexyl, phenyl, etc, Representative embodiments are as follows:



Examples of ring residues for the guanidine group (W) are pyrrolidine, piperidine, morpholine thiomorpholine, piperazine, N-methylpiperazine, and tetrahydropyrimidine. In addition, G^2 may also be a lone pair of electrons. Representative embodiments are as follows.



Examples of G^1, G^2, G^3, G^4 and G^5 for the guanidine group (W) are the following: methyl, ethyl, propyl, isopropyl, cyclopropyl, butyl, isobutyl, tert-butyl, cyclobutyl, pentyl, cyclopentyl, hexyl, cyclohexyl, phenyl, etc, In addition, G^2 may also be a lone pair of electrons. Representative embodiments are as follows:



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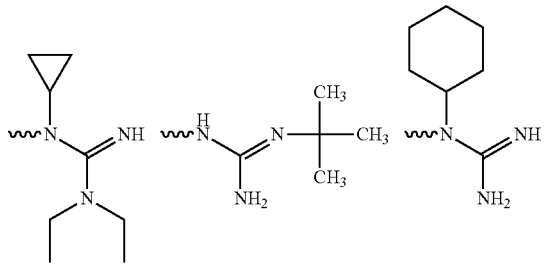
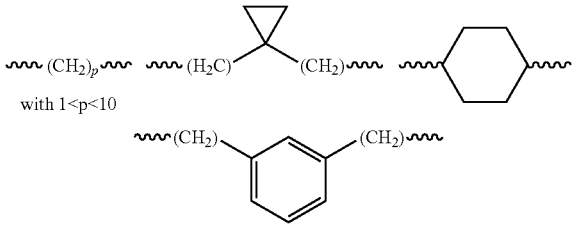
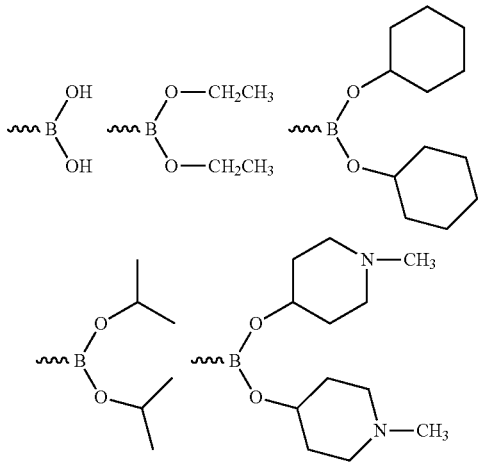


Examples of ring residues contained in X are cyclopropane, cylobutane, cyclopentane, cyclohexane or phenyl. Examples of X are shown in the following representative embodiments, where the wavy lines represent the bonds that connect X to the W part and the bore-containing part:

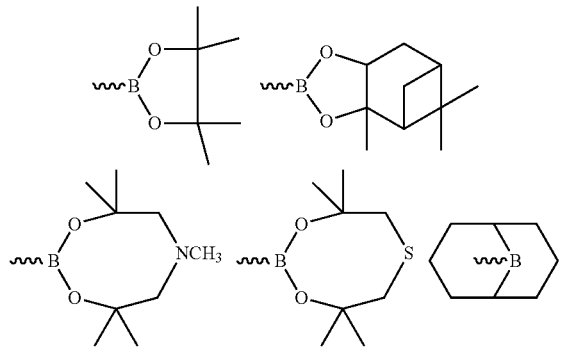


In yet another embodiment, Y and/or Z are OH or OR. When R is a cyclic alkyl group, this group optionally incorporates one or more heteroatoms such as N, O, S and halogen.

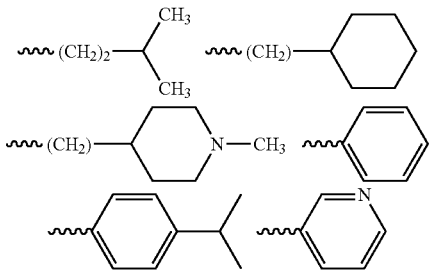
Examples of such Y and Z groups are shown in the following representative embodiments, where the wavy lines represent the bond connecting the boron atom to the other part of the molecule:



In yet another embodiment, Y and Z are branches of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms. Examples of such embodiments are shown below. The wavy line represents the bond connecting the B atom to the other part of the molecule:

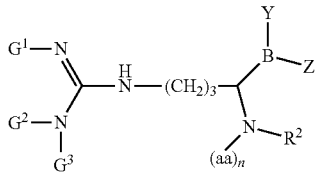


In yet another embodiment, Z is a linear or branched or cyclic alkyl group containing from 1 to 15 carbon atoms. Optionally, the linear or branched alkyl group for Z is substituted with one or more heteroatoms such as N, O, S and halogen. Examples of such Z groups are shown in the following representative embodiments, where the wavy lines represent the bond connecting Z to the other part of the molecule:



In yet another embodiment, the W—X—C—N(C═O)—R^2 part of the compound having formula I or II (structure bearing the boron atom) is an amino acid residue positively charged. An amino acid residue positively charged may be selected among arginine, lysine, ornithine or a derivative of one of these three amino acid residues as defined above.

In yet another aspect, the present invention relates to a compound based on boroarginine and having the following formula III:



III

wherein

B represents a boron atom;

Y is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms;

Z is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms, or is a linear or branched or cyclic alkyl group containing from 1 to 15 carbon atoms;

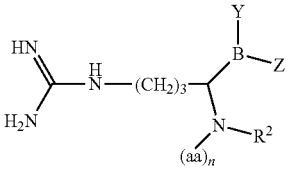
R^2 is either H, or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms, or an OR group wherein R may be H or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms;

G^1, G^2 and G^3 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates the N atom and that contains a total of up to 15 atoms; and

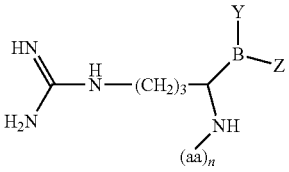
"aa" refers to any amino acyl residue or derivative thereof, and n is at least two.

Another embodiment of a compound based on boroarginine is a compound of formula IV, wherein at least one of R^2, G^1, G^2 and G^3 is H. Alternatively or in combination with the previous embodiment, at least one of R^2, G^1, G^2 and G^3 is a protecting group as defined above.

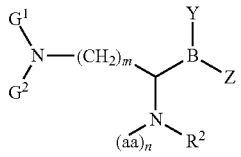
In another embodiment of a compound based on boroarginine is a compound of formula III, wherein G^1, G^2 and G^3 are H and having the following formula:



A compound based on boroarginine is a compound of formula III, wherein R^2, G^1, G^2 and G^3 are H and having the following formula:



In yet another embodiment, the compound of the invention is based on borolysine or boroornithine and has the following formula IV:



IV

wherein

B represents a boron atom;

Y is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms;

Z is an OH, or an is OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms, or is a linear or branched or cyclic alkyl group containing from 1 to 15 carbon atoms;

R^2 is either H, or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms, or an OR group wherein R may be H or a or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms;

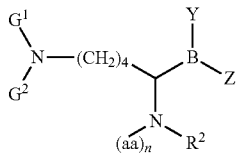
G^1 and G^2 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates the N atom and that contains a total of up to 15 atoms;

"aa" refers to any amino acid residue or derivative thereof, and n is at least two; and

m equals to 3 or 4.

In a embodiment, the compound is based on borolysine (m equals 4), and has the following formula (V)

V



wherein

B represents a boron atom;

Y is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms;

Z is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms, or is a linear or branched or cyclic alkyl group containing from 1 to 15 carbon atoms;

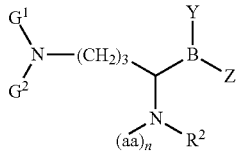
R^2 is either H, or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms, or an OR group wherein R may be H or a or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms;

G^1 and G^2 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates the N atom and that contains a total of up to 15 atoms; and

"aa" refers to any amino acid residue or derivative thereof, and n is at least two.

In another embodiment, the compound is based on boroornithine (m equals 3), and has the following formula (VI)

VI



wherein

B represents a boron atom;

Y is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms;

Z is an OH, or is an OR group wherein R is a linear, branched or cyclic alkyl group incorporating from 1 to 10 carbon atoms, or is a branch of a homocyclic or heterocyclic structure, which incorporates the B atom and which contains up to 20 atoms chosen from C, O, N, and S atoms, or is a linear or branched or cyclic alkyl group containing from 1 to 15 carbon atoms;

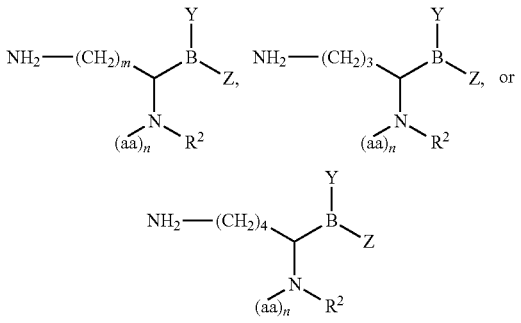
R^2 is either H, or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms, or an OR group wherein R may be H or a or a linear or branched or cyclic alkyl group containing from 1 to 10 carbon atoms;

G^1 and G^2 are, independently, H, or linear or branched or cyclic alkyl groups containing from 1 to 10 carbon atoms, or branches of a ring system that incorporates the N atom and that contains a total of up to 15 atoms; and

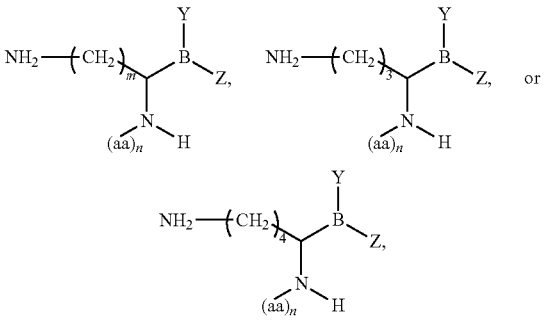
"aa" refers to any amino acid residue or derivative thereof, and n is at least two.

Another compound is a compound of formula IV, V or VI, wherein at least one of R^2, G^1 and G^2 is H. Alternatively or in combination with the previous embodiment, at least one of R^2, G^1 and G^2 is a protecting group as defined above.

Other compounds based either on formula IV, V or VI are compound wherein G^1 and G^2 are H and have the following formula:



In another embodiment of a compound based on a compound of formula IV, V or VI, wherein R^2, G^1 and G^2 are H and have the following formula:



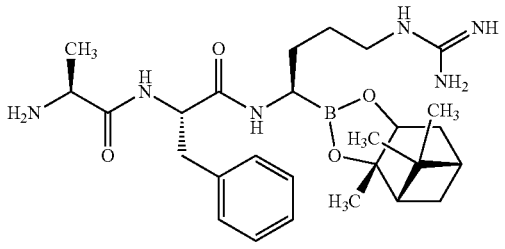
In yet another aspect, the compounds based on boroarginine (based on formula IV), on borolysine (based on formula V) or on boroornithine (based on formula VI), disclosed above, are also characterized by at least one of the following:

- at least one of Y or Z is OH. In an embodiment, Y and Z are OH.
- n is at least 2, and in another aspect is between 2 and 20, in another embodiment between 2 and 15, in another embodiment between 2 and 10, and in another embodiment between 2 and 5. In yet another aspect equals 2, 3, 4 or 5. When n equals 2, the resulting molecule is called a triboropeptide; when n equals 3, the resulting molecule is called a tetraboropeptide. The particular amino acid residues or derivative thereof that may constitute the triboropeptides or tetraboropeptides of the invention as well as the particular combination of amino acids residues have already been disclosed above for the definition of the compound of formula II, and apply in the same manner for the present triboropeptides and tetraboropeptides; and
- the first N-terminal amino acid residue of the n amino acids bears a protecting group as defined above. In another embodiment, the protecting group is linked to the free NH_2 group of the first N-terminal amino acid.

Examples of the compounds of formula I indicated are the following triboropetides and tetraboropetides: Tyr-Pro-BoroArg, Ala-Phe-BoroArg, Arg-Glu-BoroArg, Glu-Gly-BoroArg, Trp-Glu-BoroArg, Thr-Pro-BoroArg, Leu-Leu-BoroArg, Phe-Arg-Val-BoroArg, Arg-Cys-Thr-BoroArg, Gly-Cys-Pro-BoroArg, Gly-Pro-Cys-BoroArg, Gly-Cys-Asn-BoroArg and Lys-Gly-Asp-BoroArg.

Other compounds are Ala-Phe-BoroArg, Ac-Ala-Phe-BoroArg, Glu-Gly-BoroArg and Ac-Glu-Gly-BoroArg, as disclosed respectively in formulas VII, VIII, IX and X.

VII



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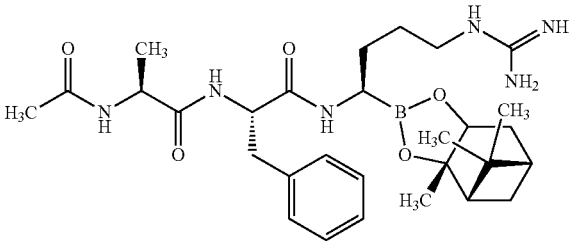
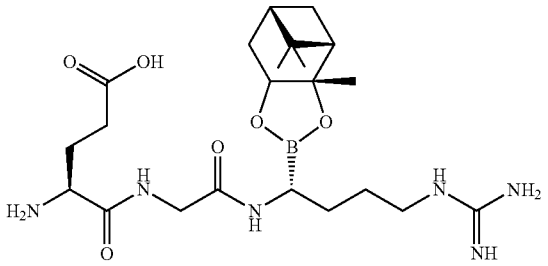
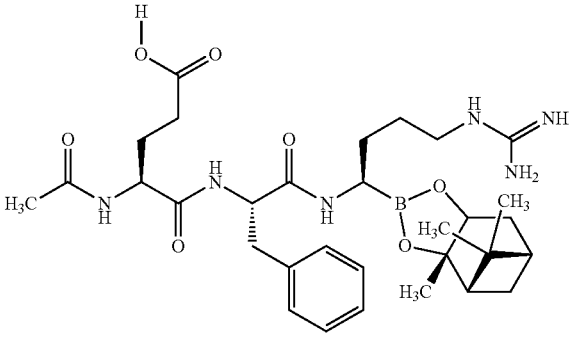
VIII



IX



X



All the compounds of the invention disclosed in the present application are under a free base form or are pharmaceutically acceptable salts thereof.

In contrast, the compounds of the invention are not at least one of the following:

Ac-(D,L)Phe-boroArg-$C_{10}N_{16}$.BSA (benzene sulfonic acid), Ac-Phe-boroOrn-$C_{10}H_{16}$.BSA, Ac-Phe boroArg-$C_{10}H_{16}$.HCl, H-(D)Phe-Pro-boroIrg-$C_{10}H_{16}$.HBr.HCl, Boc-(D)Phe-Pro-boroIrg-$C_{10}H_{16}$. HBr, Ac-Phe-boroIrg-$C_{10}H_{16}$.HBr, Ac-Ala-Lys(Boc)-boroOrn-$C_{10}H_{16}$.BSA, Ac-Ala-Lys(Boc)-boroIrg-$C_{10}H_{16}$.HBr, Boc-(D)Phe-Pro-boroArg-$C_{10}H_{16}$.BSA, Boc-(D)Phe-Phe-BoroIrg-$C_{10}H_{16}$.HBr, H-(D)Phe-Pro-boroArg-$C_{10}H_{16}$.HCl, Boc-(D)Phe-Phe-boroOrn-$C_{10}H_{16}$.BSA, Boc-(D)Phe-Phe-boroArg-$C_{10}H_{16}$.BSA, Ac-Ala-Lys (Boc)-boroArg-$C_{10}H_{16}$.BSA, Ac-(D)Phe-Pro-boroArg-$C_{10}H_{16}$.HCl, Ac-(D)Phe-Pro-boroArg-OH.HCl, Boc-Leu-Gly-Leu-Ala-boroIrg-$C_{10}H_{16}$.HBr, Boc-Leu-Gly-Leu-Ala-boroOrn-$C_{10}H_{16}$.BSA, Boc-Leu-Gly-Leu-Ala-boroArg-$C_{10}H_{16}$.BSA, Bz-Pro-Phe-boroOrn-$C_{10}H_{16}$.BSA, Bz-Pro-Phe-boroArg-$C_{10}H_{16}$.BSA, Boc-Ala-Phe-(D,L)boroIrg-$C_{10}H_{12}$.HBr, Bz-Glu(OBu)-Gly-boroIrg-$C_{10}H_{16}$.HBr, Bz-Glu-Gly-boroArg-$C_{10}H_{16}$.BSA, Bz-Glu(OBu)-Gly-boroOrg-$C_{10}H_{16}$.BSA, Bz-Glu(OBu)-Gly-boroArg-$C_{10}H_{16}$.BSA, Bz-Pro-Phe-boroIrg-$C_{10}H_{16}$.H Br, Z-Phe-Gly-Gly-boroIrg-$C_{10}H_{16}$.HBr, Boc-Ala-Phe-(D,L)borohomoIrg-$C_{10}H_{12}$.HBr, Bz-Pro-Phe-boroArg-

OH.HCl, Bz-Pro-Phe-boroArg-F, H-(D)Phe-Pro-boroArg-$C_{10}H_{16}$.2HCl, H-(D)Phe-Phe-boroArg-$C_{10}H_{16}$.2HCl, Ac-Ala-Lys-boroArg-$C_{10}H_{16}$.2HCl, H-Leu-Gly-Leu-Ala-boroArg-$C_{10}H_{16}$.HCl.BSA, Boc-Ala-Phe-(D,L)boroLys-$C_{10}H_{12}$.HCl, H-Ala-Phe-(D,L)boroLys-$C_{10}H_{12}$.2HCl, Boc-(D)Val-Leu-boroLys-$C_{10}H_{12}$.HCl, Ac-Phe-boroLys-$C_{10}H_{12}$.HCl, Bz-Glu-Gly-boroArg-$C_{10}H_{16}$.BSA, H-(D)Phe-Phe-boroIrg-$C_{10}H_{16}$.2HBr, H-Leu-Gly-Leu-Ala-boroIrg-$C_{10}H_{16}$.2HBr, H-Ala-Phe-(D,L)boroIrg-$C_{10}H_{12}$.2HBr, Bz-Glu-Gly-boroIrg-$C_{10}H_{16}$.HBr, H-Ala-Phe-(D,L)boroHomoIrg-$C_{10}H_{12}$.2HBr, Ac-Ala-Lys-boroIrg-$C_{10}H_{16}$.2HBr, Bz-boroIrg-$C_{10}H_{12}$.HBr, Bz-boroOrn-$C_{10}H_{12}$.BSA, Bz-boroArg-$C_{10}H_{12}$.BSA, Ac-Leu-Thr(OBu)-boroOrn-$C_{10}H_{16}$.BSA, Ac-Leu-Thr(OBu)boroArg-$C_{10}H_{16}$.BSA, Ac-Leu-Thr-boroArg-$C_{10}H_{16}$.BSA, Ac-Lys(Boc)-Pro-boroOrn-$C_{10}H_{16}$.BSA, Ac-Lys(Boc)-Pro-boroArg-$C_{10}H_{16}$.BSA, Ac-Lys-Pro-boroArg-$C_{10}H_{16}$.BSA, Ac-Ala-Glu(OBu)-boroOrn-$C_{10}H_{16}$.BSA, Ac-Ala-Glu(OBu)-boroArg-$C_{10}H_{16}$.BSA, Ac-Ala-Glu-boroArg-$C_{10}H_{16}$.BSA, Boc-Val-Val-boroLys-$C_{10}H_{12}$.BSA, H-Val-Va]-boroLys-$C_{10}H_{12}$.BSA.TFA, Boc-(D)Phe-Phe-boroLys-$C_{10}H_{12}$.BSA, H-(D)Phe-Phe-boroLys-$C_{10}H_{12}$.BSA.TFA, Boc-Glu-Phe-boroLys-$C_{10}H_{12}$.BSA and PyroGlu-Phe-boroLys-$C_{10}H_{12}$.BSA, disclosed in U.S. Pat. No. 5,187,157;

Ac-boroArg-OH.HCl, disclosed in Lebarbier et al.; (1998) Biorganic and Medicial Chemistry letters 8: 2573-2576;

Ac-(D)-Phe-Pro-boroArg-OH, disclosed in Quan et al.; (1997) Biorganic and Medicial Chemistry letters 7(13): 1595-1600;

Ac-Ala-Lys-boroArg-OH_2, disclosed in Holyoak et al. (2003); Biochemistry 42: 6709-6718.

Pro-Phe-BoroArg-OH, disclosed in Stadnicki et al. (1998); The FASEB Journal 12: 325-333.

Bz-Nle-Lys-Lys-boroArg-OH_2, disclosed in Yien et al. (2006); Biorganic and Medicial Chemistry letters 16: 36-39.

H-Phe-Pro-BoroArg, disclosed in Kettner et al. (1990); The Journal of Biological Chemistry 265(30): 18289-18297.

Ac-Arg-Glu-Lys-boroArg pinanediol, disclosed in Komiyama et al. (2005); Antimicrobial Agents and Chemotherapy 49(9): 3875-3882.

(BOC)-Ala-Val-Lys-boronate, disclosed in Katz et al. (1995); Biochemistry 34: 8264-8280.

The molecules of the invention are synthesized using well-know processes. As an example, FIG. **7** discloses various processes to synthesize any compound of the present invention. The examples disclosed relates to the synthesis of a borolysine tripeptide (**7**A), a bororarginine tripeptide and a boroornithine tripeptide (**7**B to **7**E). These examples may be easily applied to synthesize other compounds disclosed herein. Especially, the person skilled in the art may replace the nature and number of amino acid residues to link to the boroarginine, borolysine or boroornithine moiety.

Besides their structural feature, the compounds of the invention, and especially the tri or tetraboropeptides, have the capacity to specifically inhibit the activity of serine proteases and especially the activity of trypsin-like subtype serine proteases. In another embodiment, the compounds of the invention, and especially the tri or tetraboropeptides, have the capacity to selectively inhibit, the activity of enteropeptidase, especially mammalian enteropeptidases, and more especially human enteropeptidase. The sequence of the human enteropeptidase is disclosed in FIG. **6**.

In a second embodiment, the invention relates to a composition comprising one or more than one compound of the invention, especially 2, 3 or 4 compounds.

Such composition can take the form of a pharmaceutical composition which can be formulated using pharmaceutically acceptable carriers well known in the art, in suitable dosages.

In a particular embodiment, the composition further comprises a pharmaceutically suitable excipient or carrier and/or vehicle, when used for enteral or oral administration.

Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like.

Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize, wheat, rice, or potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions, which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

In addition to the active ingredients, these pharmaceutical compositions may also contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Pubiishing Co., Easton, Pa.).

In an embodiment of the invention, the compounds of the present invention may be administered in combination with at least one other drug, to achieve enhanced effects, for example with other drugs targeting enzymes different from the enteropeptidase. As an example, the compounds of the invention may be combined with drug(s) able to inhibit the adsorption or the metabolism of triglycerides.

In a third embodiment, the present invention relates to the use of any compound as defined above, and particularly any compound of the formula I to VI for the treatment of obesity, excess weight and diseases associated with an abnormal fat metabolism. Therefore, the compounds of the invention, particularly the compounds of the formula I to VI for use as drug, particularly for use in the treatment of obesity, excess weight and/or diseases associated with an abnormal fat metabolism, are part of the invention, as well as the use of a compound or a composition of the invention for the manufacture of a drug to treat obesity, excess weight and/or diseases associated with an abnormal fat metabolism.

The invention is also directed to a method to treat a mammal having obesity, having excess weight and/or suffering from diseases associated with an abnormal fat metabolism, comprising administrating at least one compound or a composition of the invention to a mammal in need thereof.

In an embodiment, any compound of the invention may be used to decrease the in vivo absorption of proteins. In another embodiment, any compound of the invention may be used to decrease the in vivo absorption of triglycerides. In a further embodiment, any compound of the invention may be used to decrease the in vivo absorption of proteins and the in vivo absorption of triglycerides.

In a further aspect, independently or in combination with the use of the compounds of the invention in absorption decrease, any compound of the invention may be used to decrease the food intake, i.e; to decrease the appetite (appetite blocker).

The compound or the composition of the invention may be used in dosage ranging from 10 mg to 10 g per day, or from 100 mg to 1 g per day, one or several times daily. The amount of compound(s) or the composition of the present invention may be administered in dosages according to the severity of the obesity, the amount of excess weight, the age of the mammal and/or the general health of the mammal.

The compounds or compositions of the invention are suitable for treating various forms of obesity and in particular obesity resulting from environmental causes (excessive nutrient intake and/or a sedentary lifestyle), resulting from genetic alterations (such as FTO gene), resulting from medical illness (such as hypothyroidism, Cushing's syndrome, growth hormone deficiency), resulting from smoking cessation, resulting from medications (such as steroids, atypical antipsychotics or some fertility medication), and resulting from neurological disorders.

The treatment by at least one the compounds or by the compositions of the invention of diseases associated with an abnormal fat metabolism is also contemplated. Such diseases are the following: gout disease (metabolic arthritis), type II diabetes, hyperglycemia, hyperinsulinemia, elevated blood levels of fatty acids or glycerol, syndrome X, diabetic complications, dysmetabolic syndrome and related diseases, hypercholesterolemia, atherosclerosis, hypertension, pancreatitis, hypertriglyceridemia, hyperlipidemia, stroke, coronary heart diseases, peripheral vascular diseases, peripheral arterial diseases, vascular syndromes, cholesterol-related disorders (e.g., LDL-pattern B and LDL-pattern L) and drug-induced lipodystrophy.

The invention also concerns an animal model in which the enteropeptidase gene has been inactivated. This model encompasses a mammal model (non-human) or a murine model (such as a mouse). By "inactivated", is meant that an enteropeptidase gene incorporated in this model encodes a protein having less than 50% enteropeptidase activity as compared to the wild type enteropeptidase. In one embodiment, the inactivated enteropeptidase has less than 40% enteropeptidase activity as compared to the wild type enteropeptidase. In another embodiment, the enteropeptidase of the animal model has less than 30% enteropeptidase activity as compared to the wild type enteropeptidase. In yet another embodiment, the enteropeptidase of the animal model has less than 20% or less than 10% enteropeptidase activity as compared to the wild type enteropeptidase. In still another embodiment, the inactivated enteropeptidase has no peptidase activity at all. In another aspect, the percentage of enteropeptidase activity is defined according to the conversion of trypsinogen into trypsin.

The animal model having an inactivated enteropeptidase gene may be obtained by any conventional techniques known to the person skilled in the art to obtain a knockout (KO) animal model, such as by inserting one or more amino acid substitution(s) affecting the peptidase activity of the enteropeptidase or by deleting one or several exons of the enteropeptidase gene. When the animal model is a rodent, such as a mouse, mutations may be, for example, a deletion of exons 23-28 of the prss7 gene i.e., the murine counterpart of human enteropeptidase.

The invention is also directed to the use of this animal model in drug development, for example in the screening of drugs or molecules having effects on weight. Therefore, the model can be used in a process to determine the effect of a drug or a molecule on weight comprising (a) administrating a drug to the model and (b) measuring the weight of the animal. The effect of the drug is determined by comparing the weight of the animal administered with said drug with control animals (which are administered with known molecules or with a placebo).

The expression "effect on weight" means that the drug or molecule is able to increase the weight of the animal model as compared to a placebo, or in contrast to decrease the weight of the animal model as compared to a placebo. The effect of the molecule may be observed under a low, normal or high caloric regimen.

The administration of the drug or the molecule to the animal model may be carried out orally intravenously, intraperitonally, intramuscularly, intraarterially or by sustained release systems.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention.

EXAMPLES

A. In Vitro Experiments

1. Materials

1.1. Buffer:

TN: Tris 50 mM pH=7.5 NaCl 150 mM (Tris: Euromedex Ref 26-128-3097; NaCl: Euromedex Ref 1112);

TCN: Tris 50 mM pH=7.5 NaCl 150 mM $CaCl_2$ 10 mM;

TCNB: Tris 50 mM pH=7.5 NaCl 150 mM, $CaCl_2$ 10 mM, 0.05% Brij 35 (Brij: sigma Ref B4184);

Tris 25 mM pH 8;

Pancreatic lipase Buffer: 25 mM Tris pH 9.2—0.1 mM $CaCl_2$—20 mM sodium;

Deoxycholate;

Thermolysine (Sigma, ref T7902);

Phosphoramidon disodium salt (Sigma, ref R7385);

Acetic Acid (Sigma ref A0808)

1.2. Plates

Microplate 384 small volume, clear (Greiner, ref 784101)

Microplate 384 flat bottom black (Corning, ref 3573)
Plate 96 black Nunc (VWR, ref 13634.01)
Plate 96-½ area (Corning, Ref 3695)
Microplate 96 well 800 µl unifilter (Whatman, Ref 7700-1804)

1.3. Compounds

Table I below lists four compounds of the invention (triboropeptides) and their names.

TABLE I

Name	Compound/Composition	Protection
Obe 1999	Ala-Phe-BoroArg 10 mM DMSO	No
Obe 2000	Glu-Gly-BoroArg 10 mM DMSO	No
Obe 2001	Acetyl-Ala-Phe-BoroArg 10 mM DMSO	Yes (Acetyl on the first Ala)
Obe 2002	Acetyl-Glu-Gly-BoroArg 10 mM DMSO	Yes (Acetyl of the first Glu)

1.4. Enzymes:

Table II below lists the enzymes tested for inhibition by the compounds of the invention, as well as the suppliers and the associated commercial references.

TABLE II

Enzyme	Supplier, reference
Recombinant human enteropeptidase	RD system, ref 1585SE
Trypsin from human pancreas	SIGMA, ref T6424
Thrombin from human plasma	SIGMA, ref T1063
Kallikrein from human plasma	SIGMA, ref K2638
Plasmin from human plasma	SIGMA, ref P1867
Elastase	Calbiochem, ref 324682
Chymotrypsin	Sigma, ref C8946
DPPIV (Dipeptidyl peptidase IV)	RD System, ref 1180-SE
Recombinant human Carboxypeptidase A1	RD System, ref 2856-ZN
Recombinant human Carboxypeptidase B1	RD System, ref 2897-ZN
Alpha Amylase from human pancreas	Sigma, ref A9972
Lipase	Sigma, ref L0382

1.5. Substrates:

Table III below lists the substrates used for testing the inhibition of the above enzymes by the compounds of the invention, as well as the suppliers and the associated commercial references.

TABLE III

Substrate	Supplier, reference	Corresponding enzyme
N-p-Tosyl-Gly-Pro-Arg-pNa	SIGMA, ref T1637	Enteropeptidase, Trypsin and Thrombin
H-D-Pro-Phe-Arg-pNa	Chromogenix, ref S-2302	Kallikrein
D-Ile-Phe-Lys-pNa	SIGMA, ref I6886	Plasmin
N-Succinyl-Ala-Ala-Pro-Phe-pNA	Sigma, ref S7388	Chymotrypsin
Suc-Ala-Ala-Pro-Abu-pNA	Calbiochem, ref 324699	Elastase
Ala-Pro-7-amido-4-trifluoromethylcoumarin	Calbiochem, ref 125510	DPPIV
N-(4-Methoxyphenylazoformyl)-Phe-OH potassium salt	Bachem, ref M2245	Carboxypeptidase A1
N-(4-Methoxyphenylazoformyl)-Arg-OH HCl	Bachem, ref M2525	Carboxypeptidase B1
6,8-difluoro-methylumbelliferyl octanoate (DIFMu)	InVitrogen, ref D12200	Lipase
Starch Azur	Sigma, ref S7629	Alpha Amylase

2. Protocols

2.1. Enteropeptidase Assay

Activation of the Enteropeptidase

A mix of enteropeptidase at 58.8 nM (final) and thermolysine at 1.58 ng/I (final) in TCN buffer was prepared; the mix was incubated at 37° C. during 30 minutes for activation. Phosphoramidon (10 µM final) was added to stop the activation by thermolysine.

Measurement of Enteropeptidase Activity without Inhibitor (Positive Control)

In 17 µl of TCN, 1 µl of active enzyme (2.9 nm final) and 2 µl Np Tosyl Gly Pro Arg pNa (1 mM final) were mixed, just before reading. The Absorbance was measured at 405 nm on EnVision (Perkin Elmer).

Inhibition in 384 Well Plate Small Volume (200 Final)

In 15 µl of TCN, 1 µl of active enzyme and 2 µl of Inhibitor (compound of the invention) at different concentrations were mixed, and incubated at RT (room temperature) for 30 minutes; 2 µl Np Tosyl Gly Pro Arg pNa (1 mM final) was added just before reading. The Absorbance was measured at 405 nm on EnVision (Perkin Elmer).

2.2. Thrombin, Trypsin, Kallikrein, Plasmin, Chymotrypsin and Elastase Assay

Table IV below lists the final concentrations of the tested enzymes and the corresponding substrates, in the determination of the IC_{50} without inhibitor (positive control) and in inhibition protocols:

TABLE IV

Enzyme	Susbstrate
Trypsin 10 nM	N-p-Tosyl-Gly-Pro-Arg-pNa 1 mM
Thrombin 10 nM	N-p-Tosyl-Gly-Pro-Arg-pNa 0.75 mM
Plasmin 50 nM	D-Ile-Phe-Lys-pNa 1 mM
Kallikrein 10 nM	H-D-Pro-Phe-Arg-pNa 1 mM
Chymotrypsin 50 nM	N-Succinyl-Ala-Ala-Pro-Phe-pNA 0.5 mM
Elastase 25 nM	Suc-Ala-Ala-Pro-Abu-pNA 0.5 mM

Measurement without Inhibitor (Positive Control)

In 17 µl of TN, 1 µl of enzyme and 2 µl of substrate was mixed, just before reading; The Absorbance was measured at 405 nm on EnVision (Perkin Elmer).

Inhibition in 384 Well Plate Small Volume (200 Final)

In 15 µl of TN, 1 µl of enzyme and 2 µl of Inhibitor (compound of the invention) at different concentrations were mixed and incubated at RT for 30 minutes; 2 µl of substrate was added, just before reading; The Absorbance was measured at 405 nm on EnVision (Perkin Elmer).

2.3 Carboxypeptidase A1 and B1 Assay

Activation of Carboxypeptidase A1

In 20 µl of TCNB, Carboxypeptidase A1 (100 µg/ml final) and trypsin (1 µg/ml final) were mixed and incubated at RT for 2 hours.

Activation of Carboxypeptidase B1

In 20 µl of TCNB, Carboxypeptidase B1 (100 µg/ml final) and trypsin (1 µg/ml final) were mixed and incubated at RT for 1 hour.

Table V below lists the final concentrations of the tested enzymes and the corresponding substrate, in the determination of the IC_{50} without inhibitor (positive control) and in inhibition protocols:

TABLE V

Enzyme	Susbstrate
Carboxypeptidase A1, 20 nM	N-(4-Methoxyphenylazoformyl)-Phe-OH, potassium salt 100 µM
Carboxypeptidase B1, 20 nM	N-(4-Methoxyphenylazoformyl)-Arg-OH, HCl 100 µM

Measurement without Inhibitor (Positive Control)

In 17 µl of TN, 1 µl of activated enzyme and 2 µl substrate were mixed, just before reading; The Absorbance was measured at 355 nm on EnVision (Perkin Elmer).

Inhibition in 384 Well Plate Small Volume (20 µl Final)

In 15 µl of TN, 1 µl of activated enzyme and 2 µl of Inhibitor (compounds of the invention) at different concentrations were mixed, and incubated at RT for 30 minutes; 2 µl of substrate was added, just before reading; The Absorbance was measured at 355 nm on EnVision (Perkin Elmer).

2.4. DPPIV Assay

Measurement without Inhibitor (Positive Control)

In 17 µl of Tris 25 mM pH 8, 1 µl of enzyme (1 nM final) and 2 µl of substrate (Ala-Pro-7-amido-4-trifluoromethylcoumarin, at 100 µM final) were mixed, just before reading. The Absorbance was measured on EnVision (Perkin Elmer) (Excitation 400 nm/Emission 505 nm).

Inhibition in 384 Well Plate Black (20 µl Final)

In 15 µl of Tris 25 mM pH 8, 1 µl of enzyme (1 nM final) and 2 µl of Inhibitor (compounds of the invention) at different concentrations were mixed, and incubated at RT for 30 minutes. 2 µl of substrate (Ala-Pro-7-amido-4-trifluoromethylcoumarin, at 100 µM final) was added, just before reading. The absorbance was measured on EnVision (Perkin Elmer) (Excitation 400 nm/Emission 505 nm).

2.5. Pancreatic Amylase Assay

Measurement without Inhibitor (Positive Control)

In 50 µl, 5 µl Amylase (at a starting concentration of 0.25 mg/ml) and 45 µl of Starch Azur (at a starting concentration of 2% in 20 mM NaH2PO4/50 mM Na Cl pH 7) were mixed and incubated 1 h at 37° C. with shaking. 20 µl Acetic Acid Solution (starting concentration of 2.75 M) was added, and the suspension filtered. The absorbance was measured in microplate 96 well unifilter Whatman at 595 nm.

Inhibition in 96 Well Plate Black

In 35.5 µl buffer (NaH_2PO_4/50 mM NaCl pH 7-37° C.), 5 µl of amylase (0.25 mg/ml) and 5 µl of Inhibitor (compounds of the invention) at different concentrations were mixed and incubated 30 minutes at RT. 4.5 µl of substrate (Starch Azur 20% in buffer) were added and incubated 1 hour at 37° C. under shaking. Then, 20 µl of Acetic Acid Solution (2.75 M) were added and the suspension was filtered in a microplate 96 well unifilter Whatman. Absorbance was read at 595 nm on EnVision (Perkin Elmer).

2.6. Lipase Assay

Measurement without Inhibitor (Positive Control)

In 85 µl of lipase buffer, 5 µl of lipase (56 U/ml final) and 10 µl of substrate (DiFMu at 10 µM final) were mixed, just before reading. The florescence was measured on EnVision (Perkin Elmer) (Excitation 358 nm/Emission 452 nm).

Inhibition in 96 Well Plate Black (200 Final)

In 80 µl of lipase buffer, 5 µl of lipase (56 U/ml final) and 5 µl of Inhibitor (compounds of the invention) at different concentrations were mixed, and incubated at RT for 30 minutes. Add 10 µl of substrate (DiFMu at 10 µM final) just before reading. The fluorescence was measured on EnVision (Perkin Elmer) (Excitation 358 nm/Emission 452 nm).

3. Results

As shown in FIGS. **1** to **4**, the 4 compounds (OBE1999 to OBE2000), belonging to the boropeptide family, are particularly efficient in vitro against enteropeptidase and inhibit the activity of enteropeptidase, with a high IC50 inhibition constant, at a nanomolar range from 7 to 63 nM. Moreover, these four compounds are specific of serine proteases, subtype trypsin-like, and in contrast do not inhibit chymotrypsin-like serine proteases, metallo-proteases or glucosidases (FIG. **5**).

More specifically, OBE1999 is a very good inhibitor of enteropeptidase (IC50 of 33 nM). However, it also inhibits with a good IC50 (7.5 to 29.8 nM) other enzymes such as trypsin, kallikrein and plasmin. Since the enteropeptidase is specifically located in the luminal intestine and this compound is non-absorbable, this compound is an excellent molecule to selectively inhibit the enteropeptidase, and is thus not expected to inhibit in vivo other serine protease (whose location is non-intestinal). As far OBE2001 is concerned, this compound shares the same profile as the one of OBE 1999 and therefore fulfils the same specificity and selectivity criteria.

OBE2000 is more specific for enteropeptidase and trypsin than the compounds OBE1999 and OBE2001, with low value of IC50 for enteropeptidase, and high value of IC50 not only for thrombin but also for plasmin (7100 nM) and kalikrein (260 nM). OBE2002 presents the same profile as OBE2000, except for the low IC50 value against kallikrein and plasmin as compared to OBE2000.

Consequently, these results also clearly show that all tested compounds have a remarkable efficiency in the in vitro inhibition of enteropeptidase, and can be considered as promising molecules for future in vivo experiments in animals and/or in humans. These compounds have been shown to fulfil at least two requirements necessary for treatment: the specificity of inhibition to trypsin-like subtype serine proteases, and the selectivity of the inhibition for the enteropeptidase.

B. In Vivo Experiments

1. Effect of OBE2001 on Weight

The aim of this first in vivo experiment is to determine the anti-obesity effect of OBE2001 in a model of obesity induced by a hyperlipidic diet in mice.

OBE2001 (Acetyl-Ala-Phe-BoroArg) was provided under salt form (molecular weight of 568.3 g/mol). Swiss male mice, 4 weeks of age, were divided into 2 groups of 10 animals each, receiving the following:

Group number	Diet	Dosing
1	Hight fat, ad libitum	Vehicle (water)
2	Hight fat, ad libitum	OBE2001 (40 mg/kg/day)

Administration of OBE2001 was carried out as a solution in water. Both OBE2001 or vehicle (water used a negative control) were administrated to the animals daily by the oral route in an administration of 5 ml/kg body weight.

Concerning the feeding of the animals, before the experiments (acclimation period), normal diet (reference No. 824050 SDSIDIETEX food) was available ad libitum during the acclimation period. 7 days before the beginning of the study, a hypercaloric High Fat diet (45% proteins, 4.73 Kcal/g; reference 824053 SDSIDIETEX) was given to groups 1 and 2.

Animals were weighed daily from at D-8 (8 days before the beginning of the experiment) then from D-5 to the end of the study. From D1 (first day of the experiment), food was given ad libitum.

Results

The initial body weight (in g) of mice of groups 1 and 2 at D-1 (one day prior to the experiment) as well as the gain or loss of weight according to the initial weight (for D1 to D13) are summarized in the following table and represented on FIG. **8**.

Group		D-1	D1	D2	D3	D4	D5	D6	D7
1	Mean	28.3	−2.8	−0.3	1.0	2.3	2.7	3.2	3.9
	SEM	0.7	0.2	0.1	0.2	0.3	0.4	0.4	0.5
2	Mean	28.5	−2.7	−1.2	−0.8	0.2	0.7	1.0	0.9
	SEM	0.7	0.2	0.2	0.3	0.5	0.6	0.9	1.0
	p	NS	NS	NS	NS	*	*	*	*

Group		D8	D9	D10	D11	D12	D13
1	Mean	4.4	4.7	5.1	5.4	5.9	6.3
	SEM	0.6	0.7	0.7	0.8	0.8	0.8
2	Mean	1.3	1.0	1.1	0.5	1.8	2.0
	SEM	1.3	1.3	1.4	1.5	1.0	1.1
	p	**	**	**	**	**	**

Mean: mean weight of the 10 mice per group;
SEM: standard error of the mean;
NS: non significant difference between the two groups ($p > 0.05$);
*: $p \leq 0.05$;
**: $p \leq 0.01$

As shown in FIG. **8**, this experiment showed that the weight of mice having received the OBE2001 compound is significantly lower than the weight of the control group mice. This difference is significant from day 4 and highly significant from day 8 (see Table). Consequently, the administration of OBE 2001 in mice results in the decrease of the weight as soon as day 4.

2. Effect of OBE2001 on Triglyceride Absorption

The scope of this second in vivo experiment was to analyse the effects of the OBE2001 compound on triglycerides absorption. To that purpose, mice were injected with OBE2001, before and/or after gavage with a solution enriched with cholesterol and free fatty acids (clinoleic at 20%).

In this study, 7 week old Swiss CD1 male mice were weighed and randomized for body weight; 3 groups of 5 mice were then constituted of:

- Group 1 (solvant): control group. Vehicle (H_2O+2% DMSO) at t-5 min+solution A (clinoleic 20%+cholesterol) at t0.
- Group 2 (OBE 25+25): OBE2001 25 mg/kg (before and after gavage). OBE2001 25 mg/kg (solution B) at t-5 min+OBE2001 25 mg/kg in solution A (solution C) at t0.
- Group 3 (OBE 25): OBE2001 25 mg/kg (before gavage). Vehicle (H_2O+2% DMSO) at t-5 min+OBE2001 25 mg/kg in solution A (solution C) at t0.

30 minutes before treatment (with solution A, B or C) blood was collected by retro orbital puncture after a slight anaesthesia (Isoflurane), to determine basal (t0) triglycerides levels. All solutions were then administered by oral gavage at t-5 (vehicle or solution B) or t0 (solution A or C). After administration of the solution A or C (containing fatty acids+cholesterol), blood was collected again by retro orbital puncture after a slight anaesthesia (Isoflurane) at time 60, 120, 180, 300 and 360 minutes for measurement of triglycerides levels. At time 360 minutes, mice were sacrificed.

Results:

The following values were obtained at different times after administration of the solution:

	% of basal value					
Time (min)	0	60	120	180	300	360
Group 1	100.00	227.30	298.72	203.76	109.67	89.41
Group 2	100.00	243.39	202.34	127.81	62.35	56.71
Group 3	100.00	293.17	269.24	126.45	70.22	60.92

Oral gavage with a solution of clinoleic at 20% led to an increase in triglycerides blood levels in all study groups the three first hours. Mice treated with OBE2001 at 25 mg/kg in group 2 and 3 show a decrease in triglyceride absorption as compared to the control group 1 (FIG. **9**).

Moreover, for each group, the area under the curve was calculated according to the values of the above Table.

	Area under the curve		% of decrease
Group 1	65453		
Group 2	48559	Group 2/group 1	26%
Group 3	56273	Group 3/group 1	14%

This calculation demonstrated that the overall triglycerides absorption is reduced by 26% in group 2 as compared to group 1, and of 14% in group 3 as compared to group 1. Consequently, these results demonstrate that OBE2001 is able to inhibit the enteropeptidase and reduces significantly the absorption of triglycerides in treated animals.

3. Effect of OBE2001 on Protein Absorption

This third in vivo experiment was designed to obtain information on the absorption of ^{14}C-proteins in absence or in presence of OBE2001 at two different doses (5 mg/Kg or 50 mg/Kg) after an oral administration to male Swiss mice.

15 male Swiss (CD-1) mice, 5 weeks-old and having a mean body weight of 0.021±0.002 kg were divided in 3 groups:

- Control Group (G1; solvant) containing 5 mice, received water at 5 mL/Kg at t-5 min and ^{14}C-proteins at 10 mL/Kg at T=0 min;
- Group 2 (G2) containing 5 mice (OBE 5 mg/Kg), received OBE2001 at 5 mg/Kg at t-5 min and ^{14}C-proteins at 10 mL/Kg at T=0 min;
- Group 3 (G3) containing 5^2 mice (OBE 50 mg/Kg), received OBE2001 at 50 mg/Kg at t-5 min and ^{14}C-proteins at 10 mL/Kg at T=0 min.

A preparation containing 5 ml of Clinoleic 20 (Baxter Ref DDB9500) with 1 g of glucose (Sigma; ref:G8270) and 1 g of casein (Sigma; ref: C3400) was prepared. Water was added until the solution reached 10 ml. After concentration of the radioactive solution containing the ^{14}C proteins (GE Healthcare; ref: CFA626) (×10), 200 µl of this radioactive solution was added to 2.8 ml of the preparation, in order to reach a concentration of ca 3.3 µCi/ml. The animals were starved before administration of this radioactive preparation.

The radioactive preparation was orally administered by intragastric gavage with a single dosing (ca 33.3 μCi/kg) in a volume of ca 10 ml/kg of body weight.

OBE2001 was provided in a mixture of two salts (HCl and TFA) with a 90% purity. OBE2001 was orally administered by intragastric gavage with a single dosing of 5 mg/Kg for G2 and 50 mg/Kg for G3 in a volume of ca 5 mL/kg body-weight.

Plasma aliquots were removed and placed into 6 ml pre-weighed plastic vials, at t+15, t+30, t+60, t+120 and t+240 min. About 4 ml Pico-fluor 40 was added and after shaking, the total radioactivity contained within the different samples was determined by liquid scintillation using a Packard 1900 CA spectrometer equipped with an external standard system (spectral analysis). A quenching curve for calibration purposes was set up using a ^{14}C quenched set supplied by Packard Instruments.

Liquid scintillation (LS) Counting was carried out with 2 sigma=2%, and for a maximum duration of 5 minutes (according to the method created in the 1900CA® software). Dpm (disintegration per minute) values of less than twice the background level of blank biological medium were reported as BLQ (Below the Limit of Quantitation).

Results:

During this animal experiment, no sign of morbidity or mortality occurred, and no particular effect related to dosage regimen was observed.

A Cmax of 0.9%±0.27 administered dose/g occurring at 15 min was observed for group 1 (control), while the Cmax for the treated groups tended to be lower. Indeed, for treated groups (5 mg/kg and 50 mg/kg), the Cmax were 0.58%±0.54, and 0.54%±0.24 of administered dose/g respectively. The kinetic data tended to a plateau at concentrations from 1 h to 4 h post-administration reaching respectively 0.5% administered dose/g for group 1 and 0.4% administered dose/g for groups 2 and 3 (FIG. **10**).

The AUC mean for group 1 was 2.214±0.1875% dose.h.g-1. AUC mean for two others groups was respectively 1.841±0.6402% dose.h.g-1 and 1.718±0.4366% dose.h.g-1.

This result demonstrates that protein absorption is greatly decreased in mice having received OBE2001 as compared to control mice, in the first 30 minutes following protein administration, and that this decrease is maintained during at least 4 hours.

C. Generation of a Constitutive Knock Out (KO) Mice for Enteropeptidase

In order to check that enteropeptidase is a correct target protein in counteracting obesity, a knockout (KO) mouse mimicking the enteropeptidase deficiency has been produced to first validate the target and for use as an animal model for drug development.

The generation of Constitutive Knock Out mice consists of the following steps:

1. Targeting Vector Design and Construction;
2. Targeted C57BL/6 embryonic stem (ES) cells;
3. Generation of heterozygous mice for the Constitutive Knock Out;
4. Generation of homozygous mice for the Constitutive Knock Out.

1. Targeting Vector Design and Construction

The murine counterpart of human enteropeptidase is called Prss7 and is located on mouse chromosome 16.

In order to inactivate the enteropeptidase gene, exons 23-28, which encode the catalytic domain, have been replaced by an FRT-flanked NeoR cassette. The constitutive KO allele (exons 1-22) encodes a C-terminally truncated protein which lacks the catalytic domain. The NeoR cassette has been excised through in vivo Flp-mediated recombination. Interference with the expression of the tail-to-tail positioned gene (Chodl) through this modification of the Prss7 gene cannot be excluded completely. The replacement of exons 23-28 (approximately 15 kb) with a FRT-Neo cassette (approximately 2 kb) decreases the efficiency of homologous recombination. A review of the KO allele construction is disclosed on FIG. **11**.

This targeting strategy allowed the construction of a vector comprising a constitutive enteropeptidase KO allele (sequence and scheme depicted in FIG. **12**).

2. Targeted C57BL/6 Embryonic Stem (ES) Cells

In a second step, C57BL/6N embryonic stem (ES) cells were transfected by the vector comprising the constitutive enteropeptidase KO allele by electroporation. The transformed cells were selected for their resistance to G418 and gancyclovir. Up to 251 individual ES clones were picked and 2 positive ES clones were obtained (A-D2 and A-E8). In this step, all ES Cells (ESCs) were cultured on multi-drug resistant embryonic feeder cells without antibiotics and were regularly monitored by PCR and luminometric assays for absence of contaminating bacterias and mycoplasma. Quality control (QC) also included karyotyping (chromosome count) of all expanded ES clones and lines (for an example of a publication reporting the generation of KO mice, see Roberds et al. Human molecular Genetics, 2001, Vol 10, No 12, 1317-1324).

Before being injected into blastocysts, ES cell clones were extensively validated by Southern Blot Analysis with various probes. Therefore, genomic DNA from WT cells and A-D2 or A-E8 clones was digested with AfiII, run on a gel and the transfer membrane hybridized with one of these probes, probe 5e2 (sequence: GCCGCACTATTTGCAGCATG) (FIG. **13**). The deletion of exons 23-28 of Prss7 resulted in an AfiII fragment of 11.7 kb (from position 5373 to position 17180) instead of the wild type (WT) allele of 18.8 kb (from position 5373 to position 24211).

As shown in FIG. **14**, AD-2 and A-E8 clones have a wild type allele (18.8 kb) as well as a KO allele (11.7 kb). In contrast, wild type (WT) cells have two WT (non deleted) alleles (18.8 kb).

These results confirm that the AD-2 and A-E8 clones have successfully incorporated the KO allele.

3. Generation of Heterozygous Mice for the Constitutive Knock Out

In a third step, 2 ES cell clones were injected into diploid host blastocysts, and the injected blastocyst transferred in pseudopregnant recipients (microoperation under specific pathogen free (SPF) conditions). Therefore, heterozygote (>50%) coat color chimeras were generated and breeded to heterozygosity.

To control the success of this third step, offspring were genotyped by the following PCR protocol. Genomic DNA, extracted from mouse tail, was amplified simultaneously with a first set of primers (primer 1472_23 with sequence: CGTTACTATCCATCACCTAAGC and primer 1472_24 with sequence GGGAATTCAGCTGTGTCTGAAC) corresponding to the enteropeptidase KO allele, and a second set of primers (primer 1260_1: GAGACTCTGGCTACTCATCC and primer 1260_2: CCTTCAGCAAGAGCTGGGGAC) corresponding to an internal control. The size of the enteropeptidase KO allele was 412 bp (cony), and the size of the internal control was 585 bp (c).

As expected, the amplification of genomic DNA from WT mice (115696 and 115705) gave a unique band at 585 bp. In

contrast, the amplification of genomic DNA from heterozygous mice (115695, 115702, 115706, 115707 and 115708) gave two bands, one for the control allele (585 bp) and one for the KO allele (412 bp) (FIG. **15**).

4. Generation of Homozygote Pups for Enteropeptidase

In a last step, homozygote pups for enteropeptidase KO were generated.

Enteropeptidase KO heterozygotes were crossed and 46 pups were obtained. Among the newborns, 30% (14 pups) were homozygote for the enteropeptidase KO, 50% were heterozygotes (22 pups) and 20% were wild type (homozygote dominant for the enteropeptidase gene).

The presence of the KO enteropeptidase in a homozygous strain was checked by PCR analysis, from genomic DNA, extracted from mouse tail. Genomic DNA was simultaneously amplified with a first set of primers (primer 1472_23 with sequence: CGTTACTATCCATCACCTAAGC and primer PRRS7 with sequence ATCAAGGAATCTTGGGAGCA) corresponding to the WT enteropeptidase allele, and a second set of primers (actin forward primer: CGGAACCGCYCATTGGC and actin backward primer: ACCCACACTGTGCCCATCTA) corresponding to the actin control. The size of the enteropeptidase allele was 533 bp, and the size of the actin control was 300 bp.

As can be seen from FIG. **16**, the amplified fragments from heterozygous mice, in lanes named 115755 and 115648, present two bands, one corresponding to the actin control (300 bp) and one to the WT enteropeptidase allele (533 bp). In contrast, fragments amplified from newborns 1A, 2A, 3A, 4A and 5A has a size of 300 bp and thus correspond to the sole actin control, demonstrating that they lack a WT enteropeptidase allele.

5. Phenotypic Observations

Newborns, obtained from the crossing of enteropeptidase knockout (KO) heterozygotes, were grown during 7 days and their size compared.

As can be shown in FIG. **17**, the mouse homozygous for the enteropeptidase KO (Ho) is twice smaller than the mouse heterozygous for the enteropeptidase KO (He).

These results demonstrated that enteropeptidase is a good target to fight obesity, since its complete specific inhibition results in a significant size decrease.

SEQUENCE LISTING

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<210> SEQ ID NO 1
<211> LENGTH: 3696
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Human enteropeptidase
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (41)..(3100)

<400> SEQUENCE: 1

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                                              Met Gly Ser Lys Arg
                                              1               5

ggc ata tct tct agg cat cat tct ctc agc tcc tat gaa atc atg ttt      103
Gly Ile Ser Ser Arg His His Ser Leu Ser Ser Tyr Glu Ile Met Phe
                10                  15                  20

gca gct ctc ttt gcc ata ttg gta gtg ctc tgt gct gga tta att gca      151
Ala Ala Leu Phe Ala Ile Leu Val Val Leu Cys Ala Gly Leu Ile Ala
            25                  30                  35

gta tcc tgc ctg aca atc aag gaa tcc caa cga ggt gca gca ctt gga      199
Val Ser Cys Leu Thr Ile Lys Glu Ser Gln Arg Gly Ala Ala Leu Gly
        40                  45                  50

cag agt cat gaa gcc aga gcg aca ttt aaa ata aca tcc gga gtt aca      247
Gln Ser His Glu Ala Arg Ala Thr Phe Lys Ile Thr Ser Gly Val Thr
    55                  60                  65

tat aat cct aat ttg caa gac aaa ctc tca gtg gat ttc aaa gtt ctt      295
Tyr Asn Pro Asn Leu Gln Asp Lys Leu Ser Val Asp Phe Lys Val Leu
70                  75                  80                  85

gct ttt gac ctt cag caa atg ata gat gag atc ttt cta tca agc aat      343
Ala Phe Asp Leu Gln Gln Met Ile Asp Glu Ile Phe Leu Ser Ser Asn
                90                  95                  100

ctg aag aat gaa tat aag aac tca aga gtt tta caa ttt gaa aat ggc      391
Leu Lys Asn Glu Tyr Lys Asn Ser Arg Val Leu Gln Phe Glu Asn Gly
            105                 110                 115

agc att ata gtc gta ttt gac ctt ttc ttt gcc cag tgg gtg tca gat      439
Ser Ile Ile Val Val Phe Asp Leu Phe Phe Ala Gln Trp Val Ser Asp
        120                 125                 130
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caa aat gta aaa gaa gaa ctg att caa ggc ctt gaa gca aat aaa tcc      487
Gln Asn Val Lys Glu Glu Leu Ile Gln Gly Leu Glu Ala Asn Lys Ser
    135                 140                 145

agc caa ctg gtc act ttc cat att gat ttg aac agc gtt gat atc cta      535
Ser Gln Leu Val Thr Phe His Ile Asp Leu Asn Ser Val Asp Ile Leu
150                 155                 160                 165

gac aag cta aca acc acc agt cat ctg gca act cca gga aat gtc tca      583
Asp Lys Leu Thr Thr Thr Ser His Leu Ala Thr Pro Gly Asn Val Ser
                170                 175                 180

ata gag tgc ctg cct ggt tca agt cct tgt act gat gct cta acg tgt      631
Ile Glu Cys Leu Pro Gly Ser Ser Pro Cys Thr Asp Ala Leu Thr Cys
            185                 190                 195

ata aaa gct gat tta ttt tgt gat gga gaa gta aac tgt cca gat ggt      679
Ile Lys Ala Asp Leu Phe Cys Asp Gly Glu Val Asn Cys Pro Asp Gly
        200                 205                 210

tct gac gaa gac aat aaa atg tgt gcc aca gtt tgt gat gga aga ttt      727
Ser Asp Glu Asp Asn Lys Met Cys Ala Thr Val Cys Asp Gly Arg Phe
    215                 220                 225

ttg tta act gga tca tct ggg tct ttc cag gct act cat tat cca aaa      775
Leu Leu Thr Gly Ser Ser Gly Ser Phe Gln Ala Thr His Tyr Pro Lys
230                 235                 240                 245

cct tct gaa aca agt gtt gtc tgc cag tgg atc ata cgt gta aac caa      823
Pro Ser Glu Thr Ser Val Val Cys Gln Trp Ile Ile Arg Val Asn Gln
                250                 255                 260

gga ctt tcc att aaa ctg agc ttc gat gat ttt aat aca tat tat aca      871
Gly Leu Ser Ile Lys Leu Ser Phe Asp Asp Phe Asn Thr Tyr Tyr Thr
            265                 270                 275

gat ata tta gat att tat gaa ggt gta gga tca agc aag att tta aga      919
Asp Ile Leu Asp Ile Tyr Glu Gly Val Gly Ser Ser Lys Ile Leu Arg
        280                 285                 290

gct tct att tgg gaa act aat cct ggc aca ata aga att ttt tcc aac      967
Ala Ser Ile Trp Glu Thr Asn Pro Gly Thr Ile Arg Ile Phe Ser Asn
    295                 300                 305

caa gtt act gcc acc ttt ctt ata gaa tct gat gaa agt gat tat gtt     1015
Gln Val Thr Ala Thr Phe Leu Ile Glu Ser Asp Glu Ser Asp Tyr Val
310                 315                 320                 325

ggc ttt aat gca aca tat act gca ttt aac agc agt gag ctt aat aat     1063
Gly Phe Asn Ala Thr Tyr Thr Ala Phe Asn Ser Ser Glu Leu Asn Asn
                330                 335                 340

tat gag aaa att aat tgt aac ttt gag gat ggc ttt tgt ttc tgg gtc     1111
Tyr Glu Lys Ile Asn Cys Asn Phe Glu Asp Gly Phe Cys Phe Trp Val
            345                 350                 355

cag gat cta aat gat gat aat gaa tgg gaa agg att cag gga agc acc     1159
Gln Asp Leu Asn Asp Asp Asn Glu Trp Glu Arg Ile Gln Gly Ser Thr
        360                 365                 370

ttt tct cct ttt act gga ccc aat ttt gac cac act ttt ggc aat gct     1207
Phe Ser Pro Phe Thr Gly Pro Asn Phe Asp His Thr Phe Gly Asn Ala
    375                 380                 385

tca gga ttt tac att tct acc cca act gga cca gga ggg aga caa gaa     1255
Ser Gly Phe Tyr Ile Ser Thr Pro Thr Gly Pro Gly Gly Arg Gln Glu
390                 395                 400                 405

cga gtg ggg ctt tta agc ctc cct ttg gac ccc act ttg gag cca gct     1303
Arg Val Gly Leu Leu Ser Leu Pro Leu Asp Pro Thr Leu Glu Pro Ala
                410                 415                 420

tgc ctt agt ttc tgg tat cat atg tat ggt gaa aat gtc cat aaa tta     1351
Cys Leu Ser Phe Trp Tyr His Met Tyr Gly Glu Asn Val His Lys Leu
            425                 430                 435

agc att aat atc agc aat gac caa aat atg gag aag aca gtt ttc caa     1399
Ser Ile Asn Ile Ser Asn Asp Gln Asn Met Glu Lys Thr Val Phe Gln
        440                 445                 450
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aat gaa aca gtt aaa ttt aag gtt gct ttt aat gct ttt aaa aac aag     1495
Asn Glu Thr Val Lys Phe Lys Val Ala Phe Asn Ala Phe Lys Asn Lys
470                 475                 480                 485

atc ctg agt gat att gcg ttg gat gac att agc cta aca tat ggg att     1543
Ile Leu Ser Asp Ile Ala Leu Asp Asp Ile Ser Leu Thr Tyr Gly Ile
                490                 495                 500

tgc aat ggg agt ctt tat cca gaa cca act ttg gtg cca act cct cca     1591
Cys Asn Gly Ser Leu Tyr Pro Glu Pro Thr Leu Val Pro Thr Pro Pro
            505                 510                 515

cca gaa ctt cct acg gac tgt gga gga cct ttt gag ctg tgg gag cca     1639
Pro Glu Leu Pro Thr Asp Cys Gly Gly Pro Phe Glu Leu Trp Glu Pro
        520                 525                 530

aat aca aca ttc agt tct acg aac ttt cca aac agc tac cct aat ctg     1687
Asn Thr Thr Phe Ser Ser Thr Asn Phe Pro Asn Ser Tyr Pro Asn Leu
    535                 540                 545

gct ttc tgt gtt tgg att tta aat gca caa aaa gga aag aat ata caa     1735
Ala Phe Cys Val Trp Ile Leu Asn Ala Gln Lys Gly Lys Asn Ile Gln
550                 555                 560                 565

ctt cat ttt caa gaa ttt gac tta gaa aat att aac gat gta gtt gaa     1783
Leu His Phe Gln Glu Phe Asp Leu Glu Asn Ile Asn Asp Val Val Glu
                570                 575                 580

ata aga gat ggt gaa gaa gct gat tcc ttg ctc tta gct gtg tac aca     1831
Ile Arg Asp Gly Glu Glu Ala Asp Ser Leu Leu Leu Ala Val Tyr Thr
            585                 590                 595

ggg cct ggc cca gta aag gat gtg ttc tct acc acc aac aga atg act     1879
Gly Pro Gly Pro Val Lys Asp Val Phe Ser Thr Thr Asn Arg Met Thr
        600                 605                 610

gtg ctt ctc atc act aac gat gtg ttg gca aga gga ggg ttt aaa gca     1927
Val Leu Leu Ile Thr Asn Asp Val Leu Ala Arg Gly Gly Phe Lys Ala
    615                 620                 625

aac ttt act act ggc tat cac ttg ggg att cca gag cca tgc aag gca     1975
Asn Phe Thr Thr Gly Tyr His Leu Gly Ile Pro Glu Pro Cys Lys Ala
630                 635                 640                 645

gac cat ttt caa tgt aaa aat gga gag tgt gtt cca ctg gtg aat ctc     2023
Asp His Phe Gln Cys Lys Asn Gly Glu Cys Val Pro Leu Val Asn Leu
                650                 655                 660

tgt gac ggt cat ctg cac tgt gag gat ggc tca gat gaa gca gat tgt     2071
Cys Asp Gly His Leu His Cys Glu Asp Gly Ser Asp Glu Ala Asp Cys
            665                 670                 675

gtg cgt ttt ttc aat ggc aca acg aac aac aat ggt tta gtg cgg ttc     2119
Val Arg Phe Phe Asn Gly Thr Thr Asn Asn Asn Gly Leu Val Arg Phe
        680                 685                 690

aga atc cag agc ata tgg cat aca gct tgt gct gag aac tgg acc acc     2167
Arg Ile Gln Ser Ile Trp His Thr Ala Cys Ala Glu Asn Trp Thr Thr
    695                 700                 705

cag att tca aat gat gtt tgt caa ctg ctg gga cta ggg agt gga aac     2215
Gln Ile Ser Asn Asp Val Cys Gln Leu Leu Gly Leu Gly Ser Gly Asn
710                 715                 720                 725

tca tca aag cca atc ttc tct acc gat ggt gga cca ttt gtc aaa tta     2263
Ser Ser Lys Pro Ile Phe Ser Thr Asp Gly Gly Pro Phe Val Lys Leu
                730                 735                 740

aac aca gca cct gat ggc cac tta ata cta aca ccc agt caa cag tgt     2311
Asn Thr Ala Pro Asp Gly His Leu Ile Leu Thr Pro Ser Gln Gln Cys
            745                 750                 755

tta cag gat tcc ttg att cgg tta cag tgt aac cat aaa tct tgt gga     2359
Leu Gln Asp Ser Leu Ile Arg Leu Gln Cys Asn His Lys Ser Cys Gly
        760                 765                 770
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aat gcc aaa gaa ggg gcc tgg ccc tgg gtt gtg ggt ctg tat tat ggc     2455
Asn Ala Lys Glu Gly Ala Trp Pro Trp Val Val Gly Leu Tyr Tyr Gly
790                 795                 800                 805

ggc cga ctg ctc tgc ggc gca tct ctc gtc agc agt gac tgg ctg gtg     2503
Gly Arg Leu Leu Cys Gly Ala Ser Leu Val Ser Ser Asp Trp Leu Val
                810                 815                 820

tcc gcc gca cac tgc gtg tat ggg aga aac tta gag cca tcc aag tgg     2551
Ser Ala Ala His Cys Val Tyr Gly Arg Asn Leu Glu Pro Ser Lys Trp
            825                 830                 835

aca gca atc cta ggc ctg cat atg aaa tca aat ctg acc tct cct caa     2599
Thr Ala Ile Leu Gly Leu His Met Lys Ser Asn Leu Thr Ser Pro Gln
        840                 845                 850

aca gtc cct cga tta ata gat gaa att gtc ata aac cct cat tac aat     2647
Thr Val Pro Arg Leu Ile Asp Glu Ile Val Ile Asn Pro His Tyr Asn
    855                 860                 865

agg cga aga aag gac aac gac att gcc atg atg cat ctg gaa ttt aaa     2695
Arg Arg Arg Lys Asp Asn Asp Ile Ala Met Met His Leu Glu Phe Lys
870                 875                 880                 885

gtg aat tac aca gat tac ata caa cct att tgt tta ccg gaa gaa aat     2743
Val Asn Tyr Thr Asp Tyr Ile Gln Pro Ile Cys Leu Pro Glu Glu Asn
                890                 895                 900

caa gtt ttt cct cca gga aga aat tgt tct att gct ggt tgg ggg acg     2791
Gln Val Phe Pro Pro Gly Arg Asn Cys Ser Ile Ala Gly Trp Gly Thr
            905                 910                 915

gtt gta tat caa ggt act act gca aac ata ttg caa gaa gct gat gtt     2839
Val Val Tyr Gln Gly Thr Thr Ala Asn Ile Leu Gln Glu Ala Asp Val
        920                 925                 930

cct ctt cta tca aat gag aga tgc caa cag cag atg cca gaa tat aac     2887
Pro Leu Leu Ser Asn Glu Arg Cys Gln Gln Gln Met Pro Glu Tyr Asn
    935                 940                 945

att act gaa aat atg ata tgt gca ggc tat gaa gaa gga gga ata gat     2935
Ile Thr Glu Asn Met Ile Cys Ala Gly Tyr Glu Glu Gly Gly Ile Asp
950                 955                 960                 965

tct tgt cag ggg gat tca gga gga cca tta atg tgc caa gaa aac aac     2983
Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Met Cys Gln Glu Asn Asn
                970                 975                 980

agg tgg ttc ctt gct ggt gtg acc tca ttt gga tac aag tgt gcc ctg     3031
Arg Trp Phe Leu Ala Gly Val Thr Ser Phe Gly Tyr Lys Cys Ala Leu
            985                 990                 995

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Pro Asn Arg  Pro Gly Val Tyr Ala  Arg Val Ser Arg Phe  Thr Glu
        1000                1005                1010

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Trp Ile Gln  Ser Phe Leu His
        1015

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

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Gly Ala Ala Leu Gly Gln Ser His Glu Ala Arg Ala Thr Phe Lys Ile
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Thr Ser Gly Val Thr Tyr Asn Pro Asn Leu Gln Asp Lys Leu Ser Val
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Asp Phe Lys Val Leu Ala Phe Asp Leu Gln Gln Met Ile Asp Glu Ile
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Phe Leu Ser Ser Asn Leu Lys Asn Glu Tyr Lys Asn Ser Arg Val Leu
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Gln Phe Glu Asn Gly Ser Ile Ile Val Val Phe Asp Leu Phe Phe Ala
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Gln Trp Val Ser Asp Gln Asn Val Lys Glu Glu Leu Ile Gln Gly Leu
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Glu Ala Asn Lys Ser Ser Gln Leu Val Thr Phe His Ile Asp Leu Asn
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Ser Val Asp Ile Leu Asp Lys Leu Thr Thr Thr Ser His Leu Ala Thr
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Pro Gly Asn Val Ser Ile Glu Cys Leu Pro Gly Ser Ser Pro Cys Thr
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Asp Ala Leu Thr Cys Ile Lys Ala Asp Leu Phe Cys Asp Gly Glu Val
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Cys Asp Gly Arg Phe Leu Leu Thr Gly Ser Ser Gly Ser Phe Gln Ala
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Thr His Tyr Pro Lys Pro Ser Glu Thr Ser Val Val Cys Gln Trp Ile
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Ile Arg Val Asn Gln Gly Leu Ser Ile Lys Leu Ser Phe Asp Asp Phe
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Asn Thr Tyr Tyr Thr Asp Ile Leu Asp Ile Tyr Glu Gly Val Gly Ser
275 280 285

Ser Lys Ile Leu Arg Ala Ser Ile Trp Glu Thr Asn Pro Gly Thr Ile
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Arg Ile Phe Ser Asn Gln Val Thr Ala Thr Phe Leu Ile Glu Ser Asp
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Glu Ser Asp Tyr Val Gly Phe Asn Ala Thr Tyr Thr Ala Phe Asn Ser
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Gly Gly Arg Gln Glu Arg Val Gly Leu Leu Ser Leu Pro Leu Asp Pro
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Thr Leu Glu Pro Ala Cys Leu Ser Phe Trp Tyr His Met Tyr Gly Glu
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Lys Thr Val Phe Gln Lys Glu Gly Asn Tyr Gly Asp Asn Trp Asn Tyr
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Gly Gln Val Thr Leu Asn Glu Thr Val Lys Phe Lys Val Ala Phe Asn
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Ala Phe Lys Asn Lys Ile Leu Ser Asp Ile Ala Leu Asp Asp Ile Ser
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Leu Thr Tyr Gly Ile Cys Asn Gly Ser Leu Tyr Pro Glu Pro Thr Leu
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Val Pro Thr Pro Pro Pro Glu Leu Pro Thr Asp Cys Gly Gly Pro Phe
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Ser Tyr Pro Asn Leu Ala Phe Cys Val Trp Ile Leu Asn Ala Gln Lys
545                 550                 555                 560

Gly Lys Asn Ile Gln Leu His Phe Gln Glu Phe Asp Leu Glu Asn Ile
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Asn Asp Val Val Glu Ile Arg Asp Gly Glu Glu Ala Asp Ser Leu Leu
            580                 585                 590

Leu Ala Val Tyr Thr Gly Pro Gly Pro Val Lys Asp Val Phe Ser Thr
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Thr Asn Arg Met Thr Val Leu Leu Ile Thr Asn Asp Val Leu Ala Arg
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Gly Gly Phe Lys Ala Asn Phe Thr Thr Gly Tyr His Leu Gly Ile Pro
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Glu Pro Cys Lys Ala Asp His Phe Gln Cys Lys Asn Gly Glu Cys Val
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Asp Glu Ala Asp Cys Val Arg Phe Phe Asn Gly Thr Thr Asn Asn Asn
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Glu Asn Trp Thr Thr Gln Ile Ser Asn Asp Val Cys Gln Leu Leu Gly
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Leu Gly Ser Gly Asn Ser Ser Lys Pro Ile Phe Ser Thr Asp Gly Gly
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Pro Phe Val Lys Leu Asn Thr Ala Pro Asp Gly His Leu Ile Leu Thr
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Gly Leu Tyr Tyr Gly Gly Arg Leu Leu Cys Gly Ala Ser Leu Val Ser
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Ser Asp Trp Leu Val Ser Ala Ala His Cys Val Tyr Gly Arg Asn Leu
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Glu Pro Ser Lys Trp Thr Ala Ile Leu Gly Leu His Met Lys Ser Asn
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Leu Thr Ser Pro Gln Thr Val Pro Arg Leu Ile Asp Glu Ile Val Ile
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Asn Pro His Tyr Asn Arg Arg Arg Lys Asp Asn Asp Ile Ala Met Met
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His Leu Glu Phe Lys Val Asn Tyr Thr Asp Tyr Ile Gln Pro Ile Cys
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Leu Pro Glu Glu Asn Gln Val Phe Pro Pro Gly Arg Asn Cys Ser Ile
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Ala Gly Trp Gly Thr Val Val Tyr Gln Gly Thr Thr Ala Asn Ile Leu
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Gln Glu Ala Asp Val Pro Leu Leu Ser Asn Glu Arg Cys Gln Gln Gln
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Met Pro Glu Tyr Asn Ile Thr Glu Asn Met Ile Cys Ala Gly Tyr Glu
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Glu Gly Gly Ile Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Met
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Cys Gln Glu Asn Asn Arg Trp Phe Leu Ala Gly Val Thr Ser Phe Gly
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Vector

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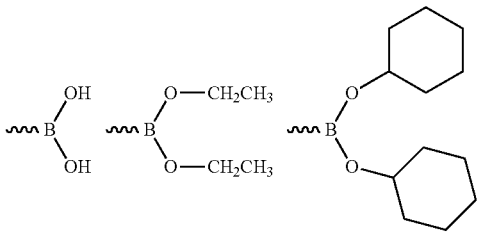
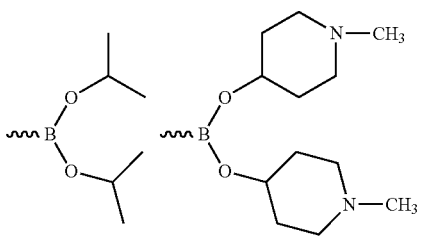
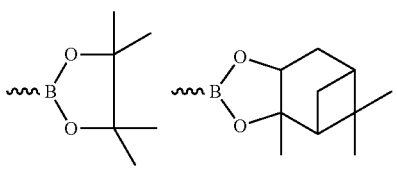
What is claimed is:

1. A method of treating a mammal having obesity or excessive weight, or a mammal suffering from abnormal fat metabolism disease selected from type II diabetes and hypertriglyceridemia, comprising the step of administering to said mammal at least one compound which inhibits the enzymatic activity of an enteropeptidase, wherein the compound is selected from the group consisting of: Glu-Gly-BoroArg, Ala-Phe-BoroArg, Glu-Gly-BoroLys, Ala-Phe-BoroLys, Ac-Glu-Gly-BoroArg, Ac-Ala-Phe-BoroArg, Ac-Glu-Gly-BoroLys, and Ac-Ala-Phe-BoroLys,

wherein Ac is acetyl, Glu is glutamic acid, Gly is glycine, Ala is alanine, Phe is phenylalanine, BoroArg is boroarginine, and BoroLys is borolysine; the BoroArg or BoroLys moieties having a functional group of selected from the group consisting of:







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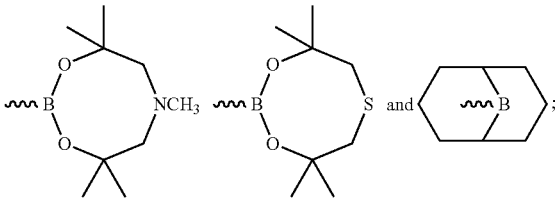
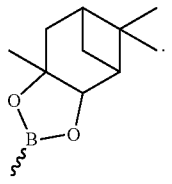
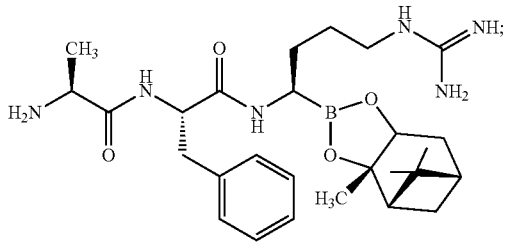


wherein the compound is non-absorbable, and selectively inhibits the enzymatic activity of an enteropeptidase localized in the intestine over other, systemically localized serine proteases.

2. The method of claim **1**, wherein the BoroArg or BoroLys moieties have a group of the formula:



3. The method of claim **1**, wherein the compound is selected from the group consisting of:



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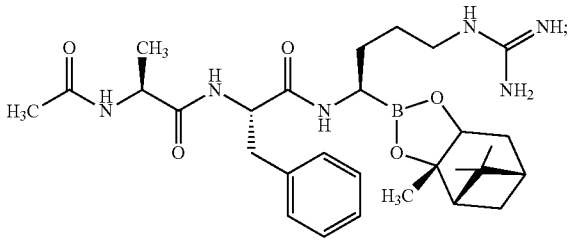

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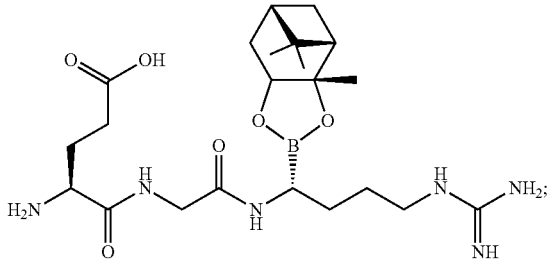

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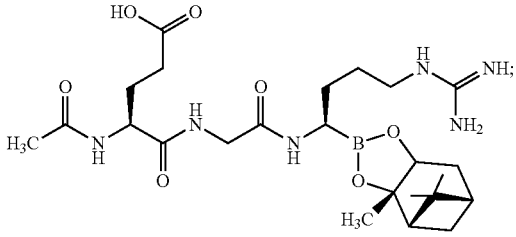

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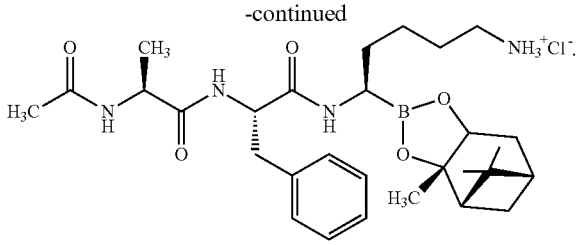

4. The method of claim **1**, wherein the compound is in a free base form or is a pharmaceutically acceptable salt thereof.

5. The method of claim **1**, wherein the Glu, Gly, Ala and Phe amino acid is each an (L)-amino acid.

6. The method of claim **1**, wherein the compound is administered in a pharmaceutical composition further comprising a pharmaceutically acceptable carrier, wherein the composition is formulated for oral or enteral administration.

7. The method according to claim **1**, wherein the compound is administered in combination with at least one other drug.

8. The method of claim **1**, wherein the mammal is a human.

9. The method of claim **1**, wherein the obesity results from environmental causes, from genetic alterations, from medical illness, from smoking cessation, from medications or from neurological disorders.

* * * * *